THE SCANDINAVIAN JOURNAL OF *Clinical & Laboratory Investigation*

EDITED FOR
THE SCANDINAVIAN SOCIETY
FOR CLINICAL CHEMISTRY
AND CLINICAL PHYSIOLOGY

Board of Editors

P. ASTRUP
H. O. BANG
E. BIERRING
E. BLEGEN
K. BRØCHNER-MORTENSEN
L. EFSKIND
L. ELDJARN
R. FRISK
N. HALLMAN
O. HELVE
C. G. HOLMBERG
B. JOSEPHSON
F.-E. KRUSIUS
J. LEHMANN
K. LUNDBÆK
P. A. OWREN
H. PALMER
A. PEKKARINEN
L. RAURAMO
F. SCHØNHEYDER
T. SJÖSTRAND
G. STRÖM
S. L. SVEINSSON
G. TÖTTERMAN

VOL. 13 æ 1961 æ NO. 3

PUBLISHED BY
MEDISINSK FYSIOLOGISK FORENINGS FORLAG
OSLO

THE SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION

Managing Editor: Svein L. Sveinsson, M. D.
Associate Editor: Einar Blegen, M. D.
Editorial Secretary: Liv Theodorsen, M. D.
Editorial Office: Central Laboratory, Ullevål Hospital, Oslo, Norway.

EDITORIAL BOARD

Editors for Denmark: *Poul Astrup*, M. D., Head of Central Laboratory, Rigshospitalet, Copenhagen.
Fritz Schønheyder, M. D., Professor of Biochemistry, University of Aarhus, Aarhus.
Editors for Finland (Suomi): *Aimo Pekkarinen*, M. D., Professor of Pharmacology, University of Turku, Turku.
Franz-Eduard Krusius, M. D., Docent, Head of Central Laboratory, Kivelä Hospital, Helsinki.
Editors for Norway: *Paul A. Owren*, M. D. Professor of Medicine, Head of Medical Dept. A, Rikshospitalet, Oslo.
Svein L. Sveinsson, M. D. Docent, Head of Central Laboratory, Ullevål Hospital, Oslo.
Editors for Sweden: *Rune Frisk*, M.D., Docent, Head of Medical Department, Ersta Sjukhus, Stockholm.
Bertil Josephson, M. D., Docent, Head of Central Laboratory, S:t Eriks Sjukhus, Stockholm.
Section on Technique and Practical Problems: *Herbert Palmer*, M.D., Head of Central Laboratory, Drammen Hospital, Drammen, Norway.

H. O. Bang, M. D., Head of Central Laboratory, City Hospital, Aalborg, Denmark.
E. Bierring, M. D., Head of Medicinsk Laboratorium, Vognmagergade 7, Copenhagen, Denmark.
Einar Blegen, M. D., Professor of Medicine, Head of Medical Department VII, Ullevål Hospital, Oslo, Norway.
K. Brøchner-Mortensen, M. D., Professor of Medicine, Head of Medical Department A, Rigshospitalet, University of Copenhagen, Denmark.
Leif Efskind, M. D., Professor of Surgery, Head of Surgical Department A, Rikshospitalet, Oslo, Norway.
Lorentz Eldjarn, M. D., Professor, Head of Central Laboratory, Rikshospitalet, Oslo, Norway.
Niilo Hallman, M. D., Professor of Pediatrics, Stenbäckinkatu 11, Helsinki, Finland.
Osmo Helve, M. D., Docent, Georgsgatan 2, Helsinki, Finland.
C. G. Holmberg, M. D., Professor, Head of Central Laboratory for Clinical Chemistry, Lasarettet, Lund, Sweden.
Jörgen Lehmann, M. D., Professor, Head of Central Laboratory, Sahlgrenska Sjukhuset, Gothenburg, Sweden.
K. Lundbæk, M. D., Professor of Medicine, Head of Medical Department M, City Hospital, Aarhus, Denmark.
Lauri Rauramo, M. D., Docent, Olavinpuisto 2 B 18, Turku, Finland.
Torgny Sjöstrand, M. D., Docent, Head of Central Laboratory of Clinical Physiology, Karolinska Sjukhuset, Stockholm, Sweden.
Gunnar Ström, M. D., Professor, Head of Central Laboratory of Clinical Physiology, University Hospital, Uppsala, Sweden.
Guido Tötterman, M. D., Professor of Medicine, Head of Medical Department, Maria Hospital, Helsinki, Finland

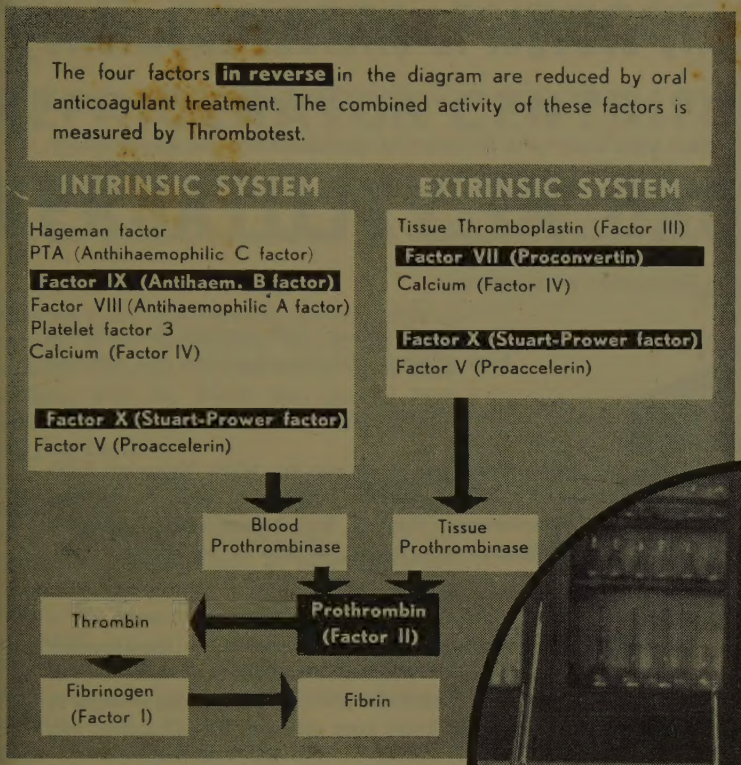
The Scandinavian Journal of Clinical and Laboratory Investigation is edited for the Scandinavian Society for Clinical Chemistry and Clinical Physiology, and will appear quarterly, 4 issues forming a volume. Subscription price D. Kr. 45.00 + porto per volume in the Scandinavian countries. Other countries D. Kr. 70.00. This price includes supplements.

The Scandinavian Journal of Clinical and Laboratory Investigation publishes original articles closely associated with clinical research and based on laboratory investigations. Pure methodological work of importance for clinical research will also be accepted for publication. Authors in the Scandinavian countries should send their manuscripts to one of the editors in their respective countries. Their addresses are printed above.

Concerning subscription apply to *E. Munksgaard, Nørregade 6, Copenhagen, Denmark.*

For advertising apply to *Jacob C. Lund, M. D., Med. kjem. laboratorium, Sentralsykehuset, Trondheim, Norway.*

The four factors **in reverse** in the diagram are reduced by oral anticoagulant treatment. The combined activity of these factors is measured by Thrombotest.



MAXIMUM SIMPLICITY AND SENSITIVITY

An important advance in the control of anticoagulant therapy

THROMBOTEST

Information, scientific documentation and supplies available through:

G. Delforge & Co., Bruxelles
Erco Läkemedel, Stockholm
Ercopharm A/S, København
Evans Medical Ltd., Liverpool
Laboratoires Delagrange, Paris
Laboratori Biochimici FISM, Milano
Charles E. Frosst & Co., Montreal
OY Medica AB, Helsinki
N.V. Pharmachemie, Haarlem
Pharmacolor AG, Basel

Includes control of intrinsic as well as extrinsic coagulation system. Reagent is stable for one year. A standard correlation curve is provided with all packs. Reproducible results from one laboratory to another. Whole blood (**capillary** or venous) is used.

No expensive equipment needed.
After initial stabilization, control required only once a month.

NYEGAARD & CO. A/S - OSLO - NORWAY

I. 251. 1. 61

automation finally brought to clinical chemistry

Already in hospitals and laboratories the world over, the Technicon Autoanalyzer is doing jobs like those listed below with an accuracy, reproducibility, and dependability, which has users standing back in awe. **The work goes faster,** up to 60 tests-per-hour, **better,** accuracy is repeatable to approximately 1%, **and cheaper,** completely automatic; no human intervention or supervision.

There's a brochure which explains the Autoanalyzer principle, detailing operation and methods.

clinical biochemical analyses

For the thousand-and-one substances for which biological fluids are analyzed . . . blood glucose, blood urea nitrogen, calcium, alkaline phosphatase, chlorides, etc. Reproducibility, approximately 1%. Elapsed time-per-test, as little as one minute.

pharmacodynamic

Sampling by direct aspiration from circulatory (or other) system of a living organ, with continuous analysis and recording of phenomena like rate absorption or excretion of drugs, changes in concentration levels of blood urea nitrogen, blood glucose, etc.

completely automatic operation

Only human participation is initial setting up of test. Autoanalyzer then takes over; introduces correct proportions of materials and reagents, passes them through the train of analytical events — mixing, dialyzing, separation, heating, color-measuring, etc. — records test results as logarithmic or easy-reading linear graphs. Selectable choice of 20, 40, or 60 separate tests-per-hour, depending on nature of test. Reproducibility, approximately 1%.

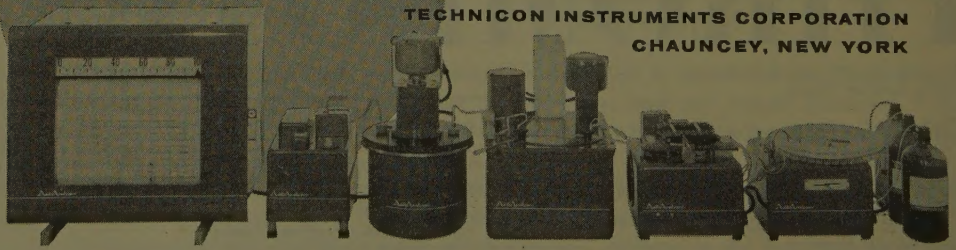
TECHNICON®

AutoAnalyzer

Trade mark

TECHNICON INSTRUMENTS CORPORATION

CHAUNCEY, NEW YORK



Exclusive Representative for Sweden: **INGENIÖRSFIRMA HUGO TILLQUIST**

Wahrendorffsgatan 8, Stockholm 7, Tel.: 24 07 90 • Kullegatan 8, Gotëborg, Tel.: 20 92 90, 20 86 10

MSE GENERAL PURPOSE REFRIGERATOR CENTRIFUGES for VERSATILITY IN CAPACITY

7 ml. TUBES TO 1250 ml. BOTTLES

Meeting users' requirements as completely as possible is the mainspring behind the range of MSE General Purpose Refrigerator Centrifuges, with the result that one of their most outstanding features is versatility in carrying capacity.

The "Major Refrigerator" Centrifuge for example has a maximum carrying capacity of 5 litres (4x1250 ml. Bottles), and it will also carry a wide variety of tube sizes—64x 15 ml. tubes or 8x100 ml. tubes among others. The three Refrigerator Centrifuges in the MSE general purpose range cater for differing *maximum* capacity needs* but all three are capable of carrying a wide choice of differing tube sizes.

These Centrifuges are in the 4-6,000 r.p.m. speed range, are fully refrigerated, and are designed to take a Superspeed Attachment for the high speed centrifuging of small quantities at speeds between 12,000 and 20,000 r.p.m.

*

For full details of carrying capacity, speeds and "g" values of the MSE "Major," "Magnum" and "Medium" Refrigerator Centrifuges please write for illustrated Catalogue.

MSE

REPRESENTATIVES:

DENMARK	Buch & Holm A.S., København K. Sølvgade 34—36
FINLAND	Kemistien Oy, Fabianinkatu 27 B, Helsinki
NORWAY	C. Grindvold, Tidemandsgt. 16, Oslo
SWEDEN	Axel Kistner, Lästmakargatan 22—24, Stockholm. Also at Göteborg and Malmö

MEASURING & SCIENTIFIC EQUIPMENT LTD., SPENSER ST., LONDON, S. W. 1. ENGLAND

Leitz

Optiska instrument

Monokulära och binokulära mikroskop
i nyaste utförande med inbyggd belysning för genom- och påfallande ljus.

Faskontrastanordning

Storfältplanoptik

Stereomikroskop

Mikrofotografiska anordningar

Mikrotomer
för framställning av finaste mikroskopiska snitt

Fotometer

Polarimeter Refraktometer
Spektroskop

Småbildkameran LEICA
med kompletteringsanordningar för vetenskaplig och teknisk fotografi

Kataloger och prospekt på begäran

Optiska instrument
av märke **ERNST LEITZ WETZLAR**
åtnjuta inom vetenskap och teknik obestritt anseende för högsta precision och tillförlitlighet. Generationers fackkunskap och ständig vetenskaplig utveckling är även i framtiden den säkra grunden för varje LEITZ-produkt.

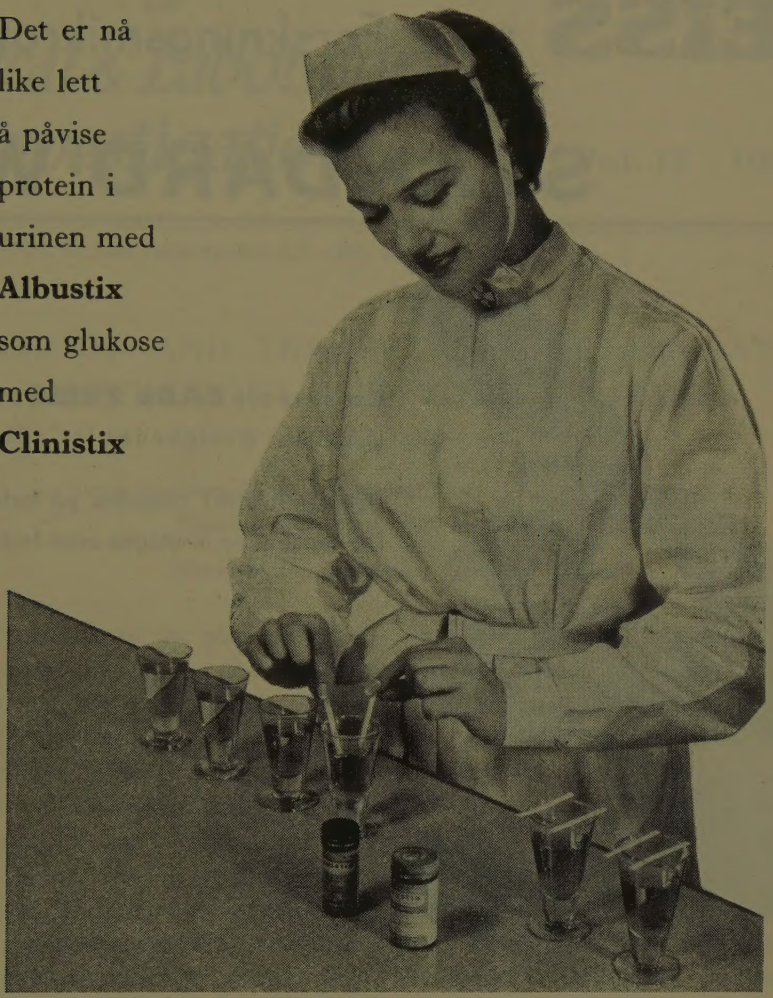
Generalagent sedan 1907:

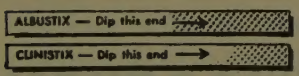
AXEL LUNDQVIST AB

Drottninggatan 28 · Stockholm · Tel. 10 87 30

Egen serviceverkstad

Det er nå
like lett
å påvise
protein i
urinen med
Albustix
som glukose
med
Clinistix





gir begge resultat samtidig

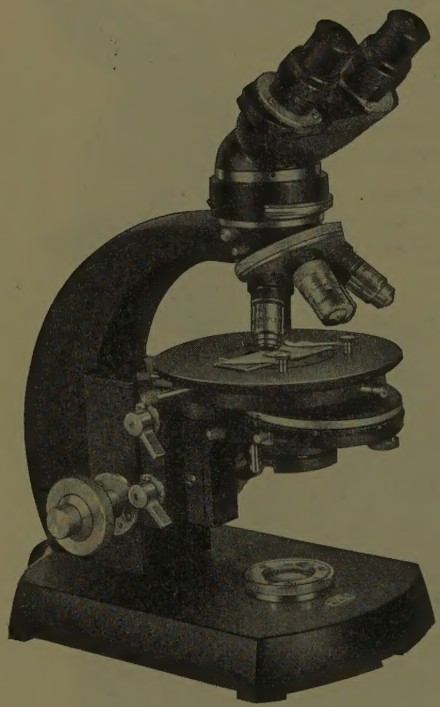
Fåes på apotek, i drogeforretninger
og medisinske varehus

AMES COMPANY (London) Ltd. LONDON

ENEIMPORTØR: IVAR SCHJETLEIN — P. B. 1612 — OSLO

ZEISS *nye* Forskningsmikroskop

STANDARD WL



Det store nye **CARL ZEISS** mikroskop
med alle muligheder for udbygning.

Specialprospekt tilsendes på forlangende.
Demonstration foretages uden forbindende.

CARL
ZEISS

Vi står til Deres tjeneste:

ZEISS SVENSKA AB
Kungsgatan 33, Stockholm C
Telf. 24 02 00

BROCK & MICHELSEN
Vestergade 23, København K
Telf. 56 31

LORENTZ E. GJERSØE A/S
Kongensgate 2, Oslo
Telf. 42 54 16

THE
SCANDINAVIAN JOURNAL
OF
Clinical & Laboratory Investigation

Vol. 13 · 1961 · No. 3

Scandinav. J. Clin. & Lab. Investigation 357—368, 13, 1961.

ALBUMIN AND TRANSFERRIN METABOLISM IN INFECTIOUS AND TOXIC DISEASES

By S. JARNUM and N. A. LASSEN

From the Department of Medicine B and of Clinical Biochemistry, Bispebjerg Hospital, Copenhagen, Denmark.

(Received for publication December 1, 1960)

The concentration of the plasma proteins changes in acute and chronic diseases which influence the organism as a whole. The increased sedimentation rate of the erythrocytes, reflecting one aspect of these changes, has been known for a number of years and remains one of our best clinical indicators of the degree of the reaction of the organism against disease. The increased sedimentation rate is an entirely unspecific phenomenon giving no indication of the nature of the disease process. In some cases the disease process is infectious while in other, *e. g.* patients with malignant tumors, nothing precise is known regarding the mechanism of the change in plasma protein concentrations. In such cases it is customary to talk of a *toxic* condition in the sense that signs of a general body reaction to disease are found. In cases of malignant tumors one could postulate that a toxin from the tumor mediated this reaction but the existence of such tumor toxin is entirely speculative.

The following changes of the concentrations of plasmaproteins are characteristic of the infectious and toxic diseases. An increased concentration of fibrinogen and several globulin fractions, α_1 and α_2 globulins especially in the more chronic and active syndromes, β_2 and γ-globulins especially in the more chronic less active syndromes. The albumin concentration is reduced as is also the concentration of the iron binding protein transferrin as evidenced by a reduction of the total iron binding capacity of plasma (Laurell, 1947). Since transferrin is a β_1 globulin comprising normally about half of the β_1 globulins in serum it is noteworthy that the β_1 fraction is the only globulin fraction present in subnormal amount in infectious and toxic diseases.

The purpose of this communication is to

Table I. *Clinical data in 15 patients with infectious and toxic diseases and in 17 control observations.*

Case	Sex	Age yrs	Height cm	Weight kg	Diagnosis	SR[1] mm/h	fever[2]	Htc.	Serum iron µg/100 ml	TIBC[3] µg/100 ml
K.H.	♂	62	163	43	Chr. polyarthr.	114	—	30	43	294
H.P.	♀	68	157	57	Chr. polyarthr.	127	+	33	24	185
E.H.	♀	67	152	63	Chr. polyarthr.	72	(+)	34	51	268
K.K.	♀	68	159	59	Chr. pyelonefr.	147	+	25	32	250
I.J.	♀	69	155	62	Chr. pyelonefr.	140	+	29	18	191
C.M.	♂	64	164	40	Chr. bronchitis	32	—	42	167	236
T.P.	♀	33	157	50	Adenitis tub.	54	—	36	54	276
A.V.	♀	68	167	60	Chr. Cholecyst.	105	(+)	37	31	198
K.B.	♀	43	165	53	Pneumonia	58	++	34	73	212
G.P.	♀	41	149	41	Lup. eryth. diss.	105	(+)	34	50	227
E.B.	♀	54	159	60	Erythema nodos.	88	(+)	38	43	222
H.R.	♂	51	175	79	Coronary occl.	65	—	41	40	256
J.P.	♀	63	155	63	Myelomatosis	134	—	36	54	224
Å.S.	♂	70	167	57	C. pulmonis	88	(+)	29	28	191
E.R.	♂	71	163	71	C. pleurae	107	(+)	37	31	271
Mean						96		34.3	50	233
No of cases			15	15	15	15	15	15	15	15
S.D.						35		4.6	35	35
H.J.	♂	81	162	62	CONTROL	9	—	46	104	295
V.T.	♂	44	168	65	CONTROL	21	—	47	88	310
M.V.	♂	73	164	80	CONTROL	5	—	43	102	283
H.S.	♂	42	174	70	CONTROL	12	—	43	94	294
W.P.	♀	73	150	36	CONTROL	36	—	35	88	336
C.J.	♂	68	175	69	CONTROL	7	—	43	109	352
H.E.J.	♂	41	165	58	CONTROL	4	—	46	138	364
J.A.	♂	49	178	84	CONTROL	16	—	44	86	282
H.R.	♂	60	165	59	CONTROL	17	—	46	107	321
H.T.	♂	41	167	62	CONTROL	7	—	43	109	352
J.P.	♂	54	182	80	CONTROL	2	—	50	84	358
S.P.	♂	61	172	74	CONTROL	20	—	40	—	—
B.J.	♂	22	163	51	CONTROL	2	—	48	—	—
O.O.	♂	21	178	66	CONTROL	2	—	40	—	—
F.	♂	50	170	62	CONTROL	—	—	39	—	—
N.A.	♂	23	172	67	CONTROL	4	—	40	—	—
E.M.	♀	42	162	54	CONTROL	8	—	41	—	—
Mean						11		43.2	101	322
No of cases						16		17	11	11
S.D.						9		3.8	16	31

[1]) Erythrocyte sedimentation rate.

[2]) ++: about 39° C, +: about 38° C, (+) about 37,5° C, —: no fever.

[3]) Total iron binding capacity of serum.

present and discuss data regarding the metabolism of albumin and transferrin labelled with radioactive iodine in infectious and toxic diseases in an attempt to elucidate the mechanism of the low plasma concentrations of both proteins.

METHODS

32 hospitalized subjects were studied, the diagnoses and some clinical data are given in Table I. 15 suffered from various infectious or toxic diseases and they all had low serum albumin and serum transferrin concentrations. 11 of these patients had infectious or infectious-like diseases. 3 had chronic polyarthritis, 2 chronic pyelonephritis, while 6 suffered from chronic bronchitis, tuberculous adenitis, chronic cholecystitis, acute severe pneumonia, disseminated lupus in active phase, and erythema nodosum respectively. Toxic disease states were present in 4 patients with the following clinical conditions: acute very severe coronary thrombosis with a protracted clinical course, myelomatosis, pulmonary neoplasma, and malignant pleural effusion.

Chronic liver disease was not present in any of the patients with infectious-toxic diseases. In some of the more acute disease states moderate abnormalities of laboratory tests of the liver function were noted, but only one patient had distinct clinical signs of a transient acute liver dysfunction as manifested by a slight degree of jaundice lasting about one week. This patient was the one suffering from a severe pneumonia caused by *staphyloccoccus aureus*. The patient was critically ill for many days and was the only one who had a high fever during the period of study.

The serum protein fractions determined by paper electrophoresis showed the characteristic changes of acute and chronic disease conditions mentioned in the introduction.

When selecting the patients special attention was paid to the degree of steady-state of the disease process, the stability of the erythrocyte sedimentation rate and the serum protein fractions used as an index of the steadiness of the disease state.

17 control subjects were also studied. These controls were just as the patients confined to bed during the study. They did not suffer from diseases affecting the plasma proteins. 8 suffered from dyspepsia or peptic ulceration (V.T., H.S., C.J., H.E.J., H.T., B.J., O.O., and N.A.); 4 had muscular pains or vertebral disc disease (J.A., F., E.M., and J.P.); 4 had chronic arteriosclerotic heart or brain disorders (H.J., M.V., S.P., and H.R.); 1 had arthrosis of the hip joint due to congenital hip luxation (W.P.). This last mentioned patient was the only of the control subjects who had a moderate elevation of the erythrocyte sedimentation rate, the cause of which was not detected. This 73-years old woman weighing only 36 kg was included in the control series as were also other elderly and frail subjects since it was considered desirable to compare the patients with infectious-toxic diseases with subjects of similar age and general constitution and not only to young healthy subjects.

The serum iron concentration in µg per 100 ml serum was measured spectrophotometrically using o-phenantroline as described by Heilmeyer & Ploetner (1937). The total iron-binding capacity in µg per 100 ml serum was measured according to the principles described by Laurell (1947) by incubating serum at pH 7.5 for 5 minutes with ferric ions added in excess of the iron-binding capacity, filtering the serum through a column of aluminum hydroxide, and determining the iron concentration of the effluent plasma. The serum transferrin concentration in g/100 ml of serum was calculated by multiplying the total iron-binding capacity by a factor of 0.0008 (Koechlin 1952). This indirect method of transferrin analysis (cf. Laurell 1947) was considered to be reliable since no cases of severe acute hepatic failure were included in the present series. The total serum protein concentration was measured in g per 100 ml using a Kjeldahl technique and the concentration of albumin and the various globulin fractions was obtained by multiplying this value by the factors indicating their relative distribution as determined by paper electrophoresis (Laurell, Laurell & Skoog, 1956).

Electrophoretically pure preparations of human albumin[1] and transferrin[2] were labelled with I^{131} according to the technique of Veall, Pearson &

[1] State Serum Institute, Copenhagen, Denmark.
[2] Behring Werke, Marburg, W. Germany.

Hanley (1955). The iodination procedure did not add more than one iodine atom per protein molecule. The albumin was iodinated at alkaline pH and non-protein-bound I^{131} was removed by passage through anion exchange resin column. The transferrin was iodinated at pH 7.4 in a 1/15 phosphate buffer and non-protein-bound I^{131} was removed by dialysis. The I^{131}-albumin and transferrin preparation used contained more than 98 per cent of the radioactivity bound to protein.

The labelling procedure did not change the electrophoretic mobility of the two proteins and the iron-binding capacity of the transferrin was also unchanged being before as well as after the procedure about 80 percent of that found for pure recrystallized transferrin by Koechlin (1.25 µg Fe per mg transferrin).

The distribution and elimination of the labelled proteins in the patients were studied by measuring the plasma concentration and urinary excretion of radioactivity for 8 to 10 days following the intravenous injection of a known amount of radioactivity (about 20 µC per study). When both proteins were studied in the same subject transferrin was studied before albumin.

The intravascular volume of distribution (plasma volume) in liters per 1.73 m^2 surface area was taken as 0.98 times the volume of distribution calculated from the plasma sample taken 15 minutes after injection of the labelled material after correction for the surface area estimated from weight and height using the monogram of Dubois & Dubois (1915). The intravascular pool of protein in grams (per 1.73 m^2) was calculated by multiplying the plasma volume by the plasma concentration. The total pool of protein in grams (per 1.73 m^2) was calculated by multiplying the intravascular pool by 1/F. F denoting the fraction of exchangeable protein located intravascularly and calculated as described by Pearson, Veal & Vetter (1958).

The turnover of the proteins was calculated from the observed elimination of radioactivity from the plasma and the excretion in the urine. Two *relative* measures of the turnover were obtained: the fractional turnover (λ) and the metabolic clearance. λ was calculated as described by Pearson *et al.* (1958) and is the percentage of the intravascular radioactivity excreted in the urine per day. It is taken to indicate the amount of protein turned o (synthesized and broken down) per 24 hours pressed in per cent of the intravascular protein po The metabolic clearance in ml per 24 hours (1.73 m^2) was obtained by multiplying the plas volume by 1/100 x λ. This is identical to sim dividing the urinary excretion of radioactivity 24 hours by the average plasma concentration radioactivity of that day and correcting for bo surface, *i. e.* the conventional method of calculat clearances in kidney function studies. The metabo clearance denotes the volume of plasma in wh the daily amount of protein turned over is contain The *absolute* turnover of the protein in grams 24 hours (per 1.73 m^2) was obtained by multiply the clearance value and the protein concentrat of the plasma.

In four studies of albumin metabolism, errors urinary collection necessitated calculating λ and from the plasma disappearance curve alone, us the equations of Matthews (1957). Extensive perience with labelled albumin in this laborat has shown that this method of calculation gi close agreement with calculation on the basis both plasma and urine measurements. In three ca the exact amount of transferrin injected was observed and the studies were calculated using subsequently determined plasma volumes from albumin-I^{131} study.

RESULTS

Distribution of albumin and transferrin (Table II).

The serum concentrations of both prote were subnormal in the patients with fectious-toxic diseases. Thus, on the avera a 26 per cent reduction was found for plasma concentrations of both albumin transferrin. The intravascular space was same for both proteins but slightly lar spaces were found in the patients with fectious-toxic diseases than in the contr

The intravascular pools were reduced the infectious-toxic conditions. That for bumin was reduced from an average

Table II. *Albumin and transferrin distribution in 15 patients with infectious and toxic diseases and in 17 control observations.*

Case	Distribution of albumin					Distribution of transferin				
	Serum conc. g/100 ml	i.vasc. space l/1.73 m^2	Pool i.vasc. per 1.73 m^2 g	% i.v. (F)	Pool total per 1.73 m^2 g	Serum conc. g/100 ml	i.v. space l/1.73 m^2	Pool i.v. per 1.73 m^2 g	% i.v. (F)	Pool total per 1.73 m^2 g
K.H.	3.42	3.41	117	38	308	0.235	3.92	9.2	45	20.5
H.P.	2.35	3.26	77	36	214	0.148	3.44	5.1	52	9.8
E.H.	3.48	3.06	107	42	254	0.214	3.06**	6.5	48	13.6
K.K.	2.73	2.90	79	36*	220	0.200	2.80	5.6	40	14.0
I.J.	2.45	3.06	75	53	142	0.153	3.06**	4.7	39	9.0
C.M.	2.82	2.65	75	34	220	0.189	2.72	5.2	45	11.5
T.P.	3.32	2.96	98	48*	205	0.229	3.22	7.4	44	16.8
A.S.	3.00	3.09	93	36	258	0.159	2.94	4.7	42	11.1
K.B.	—	—	—	—	—	0.170	3.54	6.0	45	13.3
G.P.	2.79	2.69	75	42	179	0.182	3.36	6.1	45	13.6
E.B.	3.20	2.69	86	44	196	0.178	3.19	5.7	47	12.1
H.R.	—	—	—	—	—	0.205	3.06	6.3	48	13.0
J.P.	2.91	2.78	81	36	225	0.179	2.78	5.0	44	11.3
A.S.	2.01	3.65	73	40*	183	0.153	3.55	5.5	45	12.1
E.R	—	—	—	—	—	0.217	2.69	5.8	44	13.3
Mean	2.87	3.02	86.3	40.4	214	0.187	3.16	5.9	44.9	13.0
No of cases	12	12	12	12	12	15	15	15	15	15
S.D	0.45	0.42	14.4	5.7	43	0.028	0.36	1.4	3.3	2.8
H.J.	4.21	3.02	127	38	335	0.236	3.03	7.2	46	15.6
V.T.	3.88	3.71	144	48	300	0.248	3.36	8.3	57	14.6
M.V.	3.63	2.78	101	39	258	0.226	2.70	6.1	47	13.0
H.S.	3.47	2.81	98	42	233	0.236	3.22	7.6	43	17.7
W.P.	3.36	2.40	81	32*	253	0.269	2.40**	6.4	41	15.7
C.J.	3.48	2.81	98	33	296	0.225	2.53	5.7	39	14.6
H.E.J.	—	—	—	—	—	0.291	2.25	6.5	40	16.4
J.A.	—	—	—	—	—	0.226	2.78	6.3	44	14.3
H.R.	—	—	—	—	—	0.257	2.94	7.6	46	16.4
H.T.	—	—	—	—	—	0.282	2.64	7.5	37	20.2
J.P.	—	—	—	—	—	0.286	2.82	8.1	40	20.2
S.P.	3.48	3.35	117	40	292	—	—	—	—	—
B.J.	4.75	2.12	101	38	265	—	—	—	—	—
O.O.	4.07	3.18	129	43	302	—	—	—	—	—
F.	3.80	3.18	121	41	295	—	—	—	—	—
N.A.	4.28	2.60	111	43	258	—	—	—	—	—
E.M.	4.18	2.66	111	42	264	—	—	—	—	—
Mean	3.88	2.89	112.2	39.9	279	0.253	2.79	7.0	43.6	16.3
No of cases	12	12	12	12	12	11	11	11	11	11
S.D.	0.43	0.46	11.6	4.4	32	0.025	0.34	1.2	5.5	2.3

* calculated according to Matthews (1957).

** transferrin «space» not measured but taken to be the same as the albumin «space».

112.2 g per 1.73 m^2 to an average 86.3 g per 1.73 m^2, a reduction of 23 percent which was statistically highly significant ($p < 0.001$). For transferrin the average intravascular pool was 7.0 g per 1.73 m^2 in the controls and 5.9 in the patients, a reduction of 16 per cent which also was statistically significant ($p < 0.05$). Since the intravascular pools of both proteins made up about 40 percent of the total pools in both groups, the total exchangeable pools were also reduced in the patients with infectious-toxic diseases. It follows from this observation that the transition from a normal state to disease brings about a temporary imbalance where the rates of breakdown of the two proteins are relatively higher than the rates of synthesis. Conversely, during recovery an accumulation of the proteins takes place, synthesis temporarily exceeding breakdown.

Turnover of albumin and transferrin (Table III).

Comparing the turnover data for albumin to those of transferrin, it was found that in normal man as well as in infectious-toxic diseases the *relative* turnover rates (λ and clearance) were about twice as high for transferrin as for albumin. The results obtained in a normal man studied with both proteins are shown in Fig. 1 demonstrating the more rapid disappearance of I^{131}-transferrin than of I^{131}-albumin. A significant correlation existed in the individual patient between the relative turnover rates of albumin and transferrin (Fig. 2). In the groups as a whole as well as in the individual patient transferrin had a relative turnover rate about double that of albumin. Since the serum concentrations of albumin were almost 20 times higher than the transferrin concentration it follows that the *absolute* turnover rate was almost 10 times higher for albumin than for transferrin. Thus on the average in normal man about 10 g albumin and only 1.3 g transferrin are synthesized and broken down per 24 hours and per 1.73 square meters body surface.

Comparing the infectious-toxic diseases states to normal controls with respect to turnover rates of the two proteins it was found that the *relative* turnover rates (λ and clearance) were distinctly above normal in many of the patients (Fig. 2). Other patients had normal λ and clearance. A definite relation seemed to exist between the *relative* turnover rates and the disease activity: high values were found in coronary thrombosis (H.R.), pneumonia (K.B.), disseminated erythematous lupus in acute exacerbation (G.P.), a case of severe chronic pyelonephritis in a febrile state who died one month later of the infection — not of uremia (K.K.), chronic polyarthritis in active phase with fever and during treatment with organ gold compound (H.P.), advanced neoplastic disease (J.P., Å.S., and E.R.). Normal values were found in the patients whose diseases were in a less active state. Thus, the patients with erythema nodosum (E.B.), turberculous adenitis (T.P.), and chronic cholecystitis (A.S.) were studied recovering from only moderately severe disease states while 4 remaining patients (C.M., K.H., H.E., and I.J.) had typically chronic disease states with no obvious acute exacerbation.

The *absolute* turnover rates of the two proteins in patients and controls are shown graphically in Fig. 3 plotted against the serum concentrations. The serum concentra-

Table III. *Albumin and transferrin turnover in 15 patients with infectious and toxic diseases and in 17 control observations.*

Case	Turnover of albumin			Turnover of transferrin		
	λ % of i.v. pool per day	Clearance per 1.73 m² ml/day	Absolute per 1.73 m² g/day	λ % of i.v. pool per day	Clearance per 1.73 m² ml/day	Absolute per 1.73 m² g/day
K.H.	9.2	314	10.7	19.2	752	1.8
H.P.	10.4	339	8.0	27.1	932	1.4
E.H.	6.2	190	6.6	17.4	532	1.1
K.K.	11.9*	345	9.4	28.1	788	1.6
I.J.	7.8	238	5.8	18.5	565	0.9
C.M.	10.5	278	7.8	20.2	550	1.0
T.P.	11.9*	352	11.7	21.2	684	1.6
A.S.	6.0	185	5.6	18.3	538	0.9
K.B.	—	—	—	26.4	933	1.6
G.P.	12.9	347	9.7	28.2	948	1.7
E.B.	9.3	250	8.0	17.1	545	1.0
H.R.	—	—	—	31.4	960	2.0
J.P.	12.5	348	10.1	23.7	658	1.2
A.S.	9.0*	328	6.6	23.1	820	1.3
E.R.	—	—	—	25.2	677	1.5
Mean	9.8	293	8.3	23.0	725	1.37
No of cases	12	12	12	15	15	15
S.D.	2.3	63	2.2	4.6	163	0.35
H.J.	7.3	220	9.3	14.4	436	1.0
V.T.	9.2	341	13.2	17.1	575	1.4
M.V.	7.7	214	7.8	21.2	572	1.3
H.S.	10.1	284	9.9	19.8	638	1.5
W.P.	9.1*	218	7.3	17.7	425	1.1
C.J.	7.9	222	7.7	19.8	501	1.1
H.E.J.	—	—	—	19.8	445	1.3
J.A.	—	—	—	19.6	545	1.2
H.R.	—	—	—	20.1	591	1.5
H.T.	—	—	—	14.9	394	1.1
J.P.	—	—	—	17.6	496	1.4
S.P.	10.0	335	11.7	—	—	—
B.J.	11.0	233	11.1	—	—	—
O.O.	9.5	302	12.3	—	—	—
F.	8.9	383	10.8	—	—	—
N.A.	11.2	291	12.4	—	—	—
E.M.	8.7	231	9.7	—	—	—
Mean	9.3	265	10.3	18.4	511	1.26
No of cases	12	12	12	11	11	11
S.D.	0.9	47	2.0	2.2	79	0.19

* calculated according to Matthews (1957).

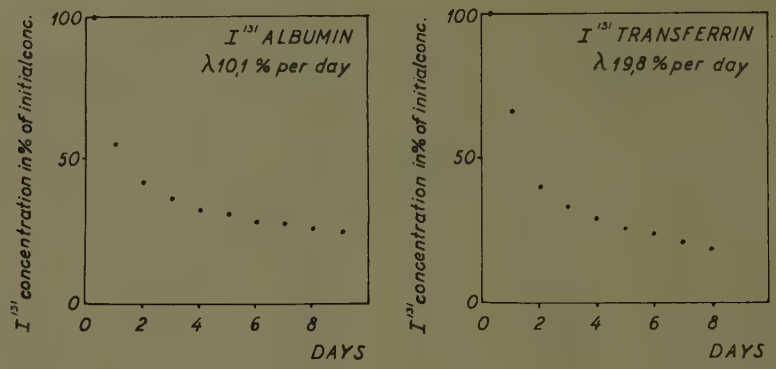


Fig. 1. I^{131}-albumin and -transferrin plasma concentrations in a normal subject (H.S., 42 years).

tions are subnormal but the turnover rates are in most cases normal or even higher than normal. The highest transferrin turnover value was 2.0 g per day which is significantly different from the control values ($p < 0.01$). This value was found in subject (H.R.) who suffered from a coronary thrombosis of one week's duration. 4 patients had low turnover values. These 4 patients all belonged to the group of clinically less active disease states mentioned above.

DISCUSSION

The distribution and elimination of I^{131}-labelled albumin has been studied extensively in man and direct experimental evidence indicates that the metabolism of the *in vitro* labelled material equals that of the genuine protein when employing an appropriate technique as shown by Mc Farlane (1957). As a precaution against systematical errors we always used each individual batch of I^{131}-albumin (and also of I^{131}-transferrin) on several subjects including normal controls as well as patients with infectious-toxic diseases. The findings in the normal subjects of a total pool of exchangeable albumin of 265 gra (per 1.73 m²) and a daily turnover of 1 grams (per 1.73 m²) are in good agreeme with the results of other investigators (B son, Yalow, Schreiber & Post, 1953) a with previous experience in this laborato (Jarnum & Schwartz, 1960).

Regarding the I^{131}-transferrin studies

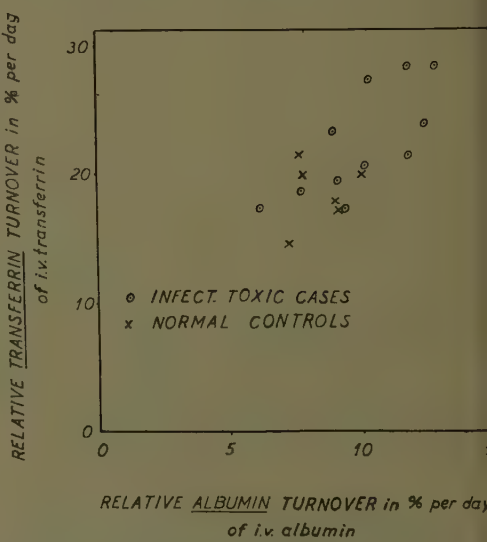


Fig. 2. The relative turnover rate (λ) of albu and transferrin.

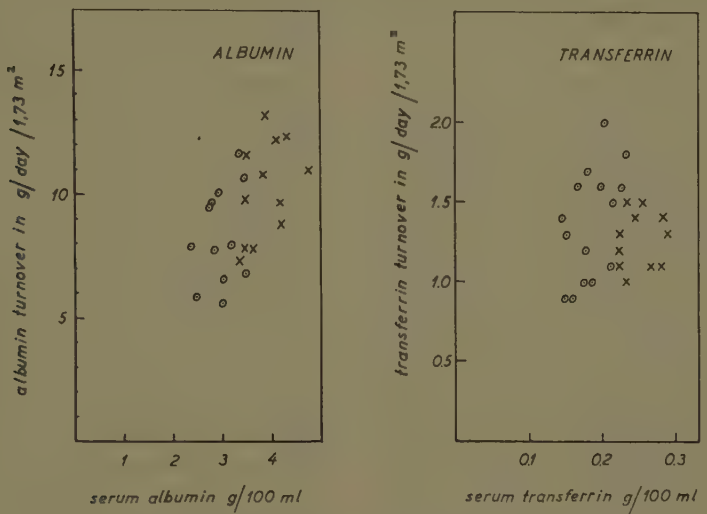


Fig. 3. Albumin and transferrin turnover in infectious and toxic diseases (⊙) and in normal controls (x).

eems reasonable to assume that the iodina-ion caused no significant denaturation of his protein since the labelling procedure ısed was even less drastic than that used for lbumin. Moreover, the electrophoretic and ellulose column chromatographic mobility f the protein was unaffected by the labelling. The iron-binding capacity was also unalter-d following iodination but it must be empha-ized that this capacity was only 80 per cent f that reported in the literature for recry-tallized transferrin (Koechlin, 1952). Thus, moderate systematic error may affect the ransferrin studies since about 20 percent of he protein labelled was non-transferrin β_1 globulins, proteins which may differ from ransferrin with respect to distribution and urnover.

On the basis of the above considerations ve conclude that the I^{131}-transferrin studies robably reflect reasonably well the meta-olism of the genuine unlabelled transferrin. Only one report has so far been published on the metabolism of labelled transferrin in man. This report, by Gitlin, Janeway & Farr (1956), was concerned with the elimination of I^{131}-labelled human transferrin in children with nephrotic syndrome where large quantities of protein-bound radioactivity were excreted in the urine. They did not report data on normal subjects in detail but mentioned that in one normal child the plasma concentration after the initial mixing of some days decreased by 50 percent in the course of at least 12 days. This value [$T\frac{1}{2}$ = at least 12 days] may be compared to that which may be calculated from our data: In normal man the average total exchangeable transferrin pool was found to be 16.3 g (per 1.73 m^2) while the daily turnover averaged 1.3 g (per 1.73 m^2). Thus the daily turnover was about 8.0 percent of the total pool, and the $T\frac{1}{2}$ of this pool can be calculated to be 0.693/0.08 = 8.7 days, which

is somewhat less than the value reported by Gitlin and coworkers (1956).

The patients with infectious and toxic diseases showed the same general abnormalities with respect to the metabolism of albumin as of transferrin. The subnormal serum concentrations were roughly parallelled by a reduction in the amount of exchangeable protein. Hence the transition from the normal state to disease involves a period of non-steady-state conditions with a relatively greater breakdown than synthesis, *i. e.* increased breakdown or decreased synthesis must occur. The steady-state studies reported in this communication can not directly demonstrate this phenomenon. However, the finding in active disease states of a normal or even slightly hypernormal absolute turnover rate strongly suggest that in acute disease it is a temporary increase in breakdown of albumin and transferrin above the level of synthesis which is responsible for the observed reduction of the amounts of exchangeable protein and low plasma concentrations.

This conclusion is in agreement with the well known increase in overall protein catabolism in acute diseases evidenced by the increased production of urea. It is possible that increased activity of the adrenal cortex is of importance in such conditions of stress for the increased catabolism of at least albumin (and possibly also transferrin) since it has been found by Grossman, Yalow & Weston (1957) that ACTH and cortisone increase the breakdown rate of albumin while the rate of synthesis remains normal or increases somewhat. These investigators also reported the result of the I^{131}-albumin metabolism in two individuals who contracted a minor febrile illness some days after injection the labelled protein. An abrupt increase the rate of albumin breakdown was observ while the rate of synthesis was unchang *i. e.* findings which are in agreement with o conclusions mentioned above. We have a observed on several occasions that even ve mild acute infectious diseases cause an ac increase of the albumin breakdown rate 20 percent. Apparently the breakdown me anism is accelerated quite readily.

In chronic less active infectious-to diseases our observations have indicated condition with low turnover rates of albu and transferrin. In such conditions no ad nocortical hyperactivity is demonstrable a the obligatory urea production is not abn mally high. Thus, in this state also a gr correlation seems to exist between ove protein metabolism (evidenced by the obli tory urea production) and the breakdo rate of specific proteins. This interest correlation was first pointed out by Davi Ricketts & Bull (1959). The mechanism the hypoalbuminemia and hypotransfer emia in the chronic less active cases may w differ from that of acute cases. If no ac disease phase initiates the condition, t perhaps a subnormal production (with relatively more normal breakdown) cau the reduction in pool sizes and plasma c centrations. In chronic conditions it see reasonable to assume that normal conditi are regained primarily by a normalizatio the production rate in combination wit relatively low breakdown.

The turnover data for albumin and tra ferrin do not, in our opinion, suggest a finite model regarding the regulation of p duction and breakdown of these proteins

or this reason we have only discussed the *bsolute* turnover rates and not the *relative* ates which may or may not represent the ctivity of the breakdown mechanism. Our ata do suggest, however, that the same eneral principles may be involved to some xtent. On a relative basis transferrin is urned over much more rapidly than albumin vhile on an absolute basis albumin turnover s much the largest, but similar deviations rom the normal state were found in the liseases studied. In cirrhosis of the liver with ypoalbuminemia, a reduced albumin turnver rate has been found (Sterling, 1951), nd similar results were obtained in two irrhotics studied with I^{131}-labelled transferin (case P.N., transferrin turnover 1.1 g/ lay, case F.S. 0.7 g/day). Also, in a patient vith protein-losing disease of the gastrointeestinal tract where the serum albumin was ow and the albumin turnover was hypernornal, the same abnormalities were found for he metabolism of transferrin.

The similarities between albumin and ransferrin metabolism suggest that the varition of the concentration of transferrin in erum may in many conditions be of an unpecific nature. Thus in infectious and toxic liseases the hypotransferrinemia would seem ot necessarily to be the effect of a primary hange in the iron metabolism. This conclusion is of some interest since it has so far een tempting to relate infectious hypotransferrinemia in some way to an abnormal iron netabolism revealed by low serum iron and nemia. That the anemia of infection is not he result of the hypotransferrinemia is indicated by the lack of anemia in patients with he nephrotic syndrome where the serum transferrin concentration is markedly reduced.

We have stressed the gross correlation between patterns of turnover of the two serum proteins studied since our data showed such a relation which has not been observed previously. This should not be taken to mean, however, that we intend to imply that the mechanisms of regulations of the two proteins are in fact identical. They are not. Thus, *e. g.* transferrin metabolism is specifically altered by iron deficiency, a condition which presumably does not influence albumin metabolism and in which Jordal (1960) has recently found relatively low values for the transferrin turnover rate.

SUMMARY

The distributions and turnover rates of radioactively labelled (I^{131}) albumin and transferrin were studied in 17 normal subjects and in 15 patients with infectious and toxic diseases.

In the normal subjects the two labelled proteins showed the same pattern of distribution, but transferrin-I^{131} disappeared twice as fast as albumin-I^{131} from the organism. Since albumin is present in a much higher concentration than transferrin, the calculated turnover rate is nevertheless considerably higher for albumin than for transferrin: An average of about 10 grams of albumin and 1.3 grams of transferrin are turned over (synthesized and broken down) per day in normal man.

In the patients with infectious-toxic diseases almost the same distribution was found as in the controls. Therefore, since the plasma concentrations are subnormal, the amounts of total exchangeable protein were

also reduced. The turnover rates of the two proteins were normal or even slightly above normal in the more acute and active disease states while they were below normal in the most inactive chronic conditions.

The results suggest that the same unspecific mechanisms are responsible for lowering the serum concentrations of albumin and transferrin in infectious-toxic diseases: a rate of synthesis which is temporarily below that of breakdown. In acute and active chronic diseases it is due to an accelerated breakdown rate (hypercatabolism), in the most chronic conditions without acute phases possibly due to a decreased rate of protein synthesis (hypoanabolism).

ACKNOWLEDGEMENT

This study has been supported by grants from "King Christian X's Foundation", and this aid is gratefully acknowledged.

REFERENCES

Berson, S. A., Yalow, R. S., Schreiber, S. S. & Post, J.: Tracer experiments with I^{131} labelled human serum albumin: distribution and degradation studies. J. Clin. Investigation *32,* 746, 1953.

Davies, J. W. L., Ricketts, C. R., and Bull, J. P.: Serum albumin catabolism and nitrogen excretion. Lancet *1,* 346, 1959.

Dubois & Dubois: Arch. intern. Med. *15,* 868, 1915. Nomogram in Documenta Geigy, Scientific Tables, 5. edition, p. 250, 1956.

Gitlin, D., Janeway, C. A. & Farr, L. E.: Studies on the metabolism of plasma proteins in the nephrotic syndrome. I. Albumin, γ-globulin, and ironbinding globulin. J. Clin. Investigation *35,* 44. 1956.

Grossman, J., Yalow, A. A. & Weston, R.: Effec of Corticotropin, hydrocortisone, and stress albumin synthesis and catabolism in man. Clin. Investigation *36,* 896, 1957 (abstracts).

Heilmeyer, L. and Ploetner, K.: Das Serumeis und die Eisenmangelkrankheit, Fischer, Je 1937.

Jarnum, S. & Schwartz, M.: Bestemmelse af alb minomsætningen med ^{131}I-mærket albun Nord. med. *63,* 708, 1960.

Jordal, R.: Personal communication, Copenhag 1960.

Koechlin, B. A.: Preparation and properties serum and plasma proteins XXVIII. The metal-combining protein of human plasma. Am. Chem. Soc. *74, 2649,* 1952.

Laurell, C. B.: Studies on the transportation a metabolism of iron in the body with special ference to the iron-binding component in man plasma. Acta physiol. Scandinav. Suppl. *46,* 1947.

— Laurell, S. & Skoog, N.: Buffer compositi in paper electrophoresis: Considerations on influence with special reference to the int action between small ions and proteins. C Chem. *2,* 99, 1956.

Matthews, C. M. E.: The theory of tracer expe ments with ^{131}I-labelled plasma proteins. Pl sics in Medicine and Biology *2,* 36, 1957.

Mc Farlane, A. S.: The behaviour of I^{131}-label plasma proteins in vivo. Ann. New York Ac Sc. *70,* 19, 1957.

Pearson, J. D., Veall, N. & Vetter, H.: A pr tical method for plasma albumin turno studies. Strahlentherap. *38,* 290, 1958. 1958.

Sterling, K.: Serum albumin turnover in Laenn cirrhosis as measured by I^{131}-tagged albun J. Clin. Investigation *30,* 1238, 1951.

Veall, N., Pearson, J. D. & Hanley, T.: The p paration of ^{132}I- and ^{131}I-labelled human ser albumin for clinical tracer studies. Brit. Radiol. *28,* 633, 1955.

ndinav. J. Clin. & Lab. Investigation 369—373, 13, 1961.

INHIBITORY ACTION OF SERUM LECITHIN ON THE EMOLYTIC ACTIVITY OF SERUM LYSOLECITHIN *IN VITRO*

By E. GJONE[1]

rom the Institute for Medical Biochemistry and Physiology, University of Oslo, Oslo, Norway.

(Received for publication November 27, 1960)

Lysolecithin has recently been isolated m lipid extracts of normal human serum silicic acid column chromatography hillips 1957, Gjone, Berry & Turner 59). The hemolytic activity of lysolecithin s represented one of the main objections the idea that this phospholipid is a normal nstituent of serum in the reported amounts. ıe present paper reports an inhibition of e hemolytic activity of lysolecithin by leci- n separated from lipid extracts of serum silicic acid chromatography.

METHODS

Extraction of lipids was carried out as described viously (Gjone *et al.* 1959).

Separation of lipids was accomplished by the use silicic acid column chromatography according to method described by Hirsch & Ahrens (1958) ng 18 grams of activated silicic acid obtained m Bio-Rad Laboratories, Richmond, California o SAB-11). Column temperature was kept con- nt at 22° C during the experiments. Columns re not reused after a completed run. After uni- m packing and dehydrating washings the column s conditioned for 12 hours with petroleum ether p. 60° to 80° C). The total lipid sample was olied in 20 ml petroleum ether. Lipids other than phospholipids were eluted from the column with 200 ml diethyl ether. Phospholipids were eluted with methanol or with chloroform-methanol mixtures. Ten ml fractions were collected by means of an adjusted siphon and an automatic fraction collector. The separation of lysolecithin was accomplished with 500 ml 1:4 (v/v) chloroform-methanol as previously described (Gjone *et al.* 1959). Rechromatography of the lecithin-sphingomyelin-fraction with 500 ml 3:2 (v/v) chloroform-methanol resulted in a satisfactory separation of these two compounds. Phosphorus was analyzed by the method of King (1932).

Identification of lipid fractions. Paper chromatography was carried out on silicic acid impregnated paper (Whatman No. 3) prepared as described by Lea, Rhodes & Stoll (1955) and using methanol-chloroform 1:4 (v/v) as eluant in the descending manner. Lecithin, sphingomyelin and lysolecithin spots were detected by immersing the chromatogram first in 2 per cent phosphomolybdic acid and then, after washings in n-butanol and water, in 0.4 per cent stannous chloride in 3 N HCl to reveal choline according to the method of Chargaff, Levine & Green (1948).

Hemolytic activity was tested by incubation with 3 ml of a 1 per cent suspension of washed human erythrocytes in isotonic saline at room temperature for 3 hours. The samples to be tested were pipetted from the respective pooled fractions after the column chromatography in chloroform/methanol solution, taken to dryness under vacuum and then dissolved in 0.1 ml methanol before the erythrocyte suspension was added. This amount of methanol

[1] Present address: Medical Department B, University Clinic, Rikshospitalet, Oslo, Norway.

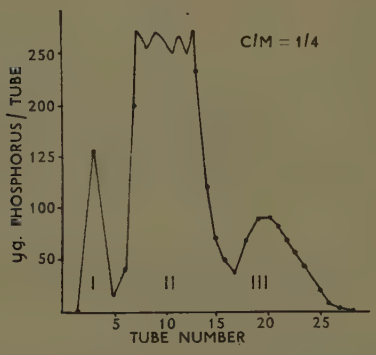


Fig. 1. Silicic acid column chromatography of phospholipids from 100 ml normal human serum. Eluant: chloroform-methanol 1:4 (c/v). 10 ml fractions.

did not alter the hemolytic properties of lysolecithin in the system used.

Standards. Syntetic L-α-dipalmytoyl-lecithin was obtained from Mann Research Laboratories, Inc., New York (lot B 3152) and Sphingomyelin from L. Light & Co. Ltd., Colnbrook, England.

RESULTS

Isolation of lysolecithin and lecithin.

Lysolecithin was isolated from lipid extracts of normal human serum by silicic acid column chromatography when phospholipid elution was accomplished with chloroform-methanol 1:4 (v/v) as shown in Fig. 1. The three peaks in order of elution have, as previously described (Gjone *et al.* 1959), been identified as I: kephalins, II: lecithin and sphingomyelin and III: lysolecithin. The peak III material was pooled and used as the serum lysolecithin in the following hemolysis experiments.

The peak II material was pooled and subjected to rechromatography on a silicic acid column using chloroform-methanol 3:2 (v/v) as eluant. A fairly distinct separation into two fractions was obtained as demonstrated in Fig. 2. Tubes 5—19 and 26—44 w pooled to give materials A and B resp tively. Descending paper chromatogra on silicic acid impregnated paper using thanol-chloroform 1:4 (v/v) as eluant carried out with materials A and B. Mate A gave one spot with Rf value of 0 whereas material B gave one spot with value of 0.33. Paper chromatography car out in the identical manner with the standards resulted in a dipalmitoyl leci spot with Rf value of 0.54 and a sphi myelin spot with Rf value of 0.34. Acc ingly, the two pooled fractions A an were identified as serum-lecithin and um-lecithin and serum-sphingomyelin spectively. Calculations of the rela amounts of phosphorus in the two peaks in good agreement with the expected va for lecithin and sphingomyelin in se These serum-lecithin and serum-sphingo lin fractions thus separated were used i following hemolysis experiments.

Hemolysis experiments.

3 ml of a 1 per cent suspension of wa human erythrocytes were used throug

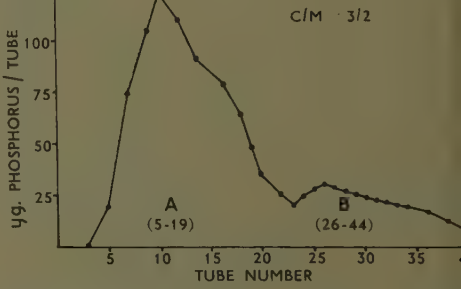


Fig. 2. Rechromatography of fraction II (F on silicic acid column. Chrloroform-methano (v/v) as eluant. 10 ml fractions.

hemolysis experiments. Hemolytic activ- was detectable with 0.01 μmole lysoleci- n and total hemolysis was observed with 0 μmole or 3 μg lysolecithinphosphorus solecithin P).

The *total phospholipid fractions* from 7 ferent sera of fasting normal humans were ted from the silicic acid columns with thanol after the elution of the nonphos- orus—containing lipids with diethyl er. The total lipid P in these sera varied m 6.25 to 11.25 mg P per 100 ml serum. mples of varying lipid content were taken t for hemolysis studies from these total ospholipid fractions. On the assumption t lysolecithin P represents approximately per cent of the total lipid P, the amounts lysolecithin P in the total phospholipid mples taken out for hemolysis studies were), 5.6, 14.4, 17.6, 20.0, 23.2, and 31.0 μg spectively. In none of the experiments with ese samples of total phospholipids of serum uld any hemolysis be observed although eir contents of lysolecithin in the isolated te would have given complete hemolysis. is experiment, therefore, demonstrates t one or more of the other phospholipids the relative amounts present in serum in- its the hemolytic activity of lysolecithin *vitro*.

Table I demonstrates the inhibitory action serum lecithin on the hemolytic activity serum lysolecithin. Degree of hemolysis given from 0 (no hemolysis) to ++++ omplete hemolysis).

The results shown in Table I indicate a oportionality between the amounts of rum lysolecithin and those of serum leci- n that completely inhibited the hemolysis *vitro*. The lecithin P:lysolecithin P ratio

Table I. *The inhibitory action of serum lecithin on the hemolytic activity of serum lysolecithin. Incubation with 3 ml 1 percent suspension of human erythrocytes in isotonic saline for 3 hours.*

Tube No	Serum lysolecithin P	Serum lecithin P	Hemolysis
1	3.0 μg	0.0 μg	++++
2	1.5 «	2.0 «	+
3	1.5 «	4.0 »	0
4	3.0 «	4.0 »	+
5	3.0 «	6.0 «	0
6	4.5 «	4.0 »	++
7	4.5 «	8.0 «	0
8	6.0 «	4.0 »	+++
9	6.0 «	6.0 «	++
10	6.0 «	8.0 «	+
11	6.0 «	12.0 «	0

in lipid extracts of normal human serum is approximately 8:1 whereas the observed ratio giving complete inhibition of the lysolecithin hemolysis *in vitro* is approximately 2:1. This inhibitority action of serum lecithin on the lysolecithin-induced hemolysis is therefore sufficient to explain why the total phospholipid fraction from normal serum did not exhibit any hemolytic activity.

The hemolytic activity of 3 μg lysolecithin P was also completely inhibited by the presence of 0.1 ml normal plasma containing approximately 6.5 μg lecithin P.

In order to study the site of the antihemolytic activity of serum lecithin, 3 μg lysolecithin P was incubated for 3 hours with 12 μg lecithin P and the erythrocyte suspension. After centrifugation for 15 minutes at 2500 rpm the clear supernatant was removed and 2.5 ml isotonic saline added together with 3 μg lysolecithin P. Complete hemolysis could be observed after 3 hours further incubation. This experiment, therefore, demonstrates

that the compound inhibiting the hemolysis had been removed with the supernatant.

Whereas serum lecithin inhibited the hemolytic action of lysolecithin in relative amounts less than that present in serum, this was not the case of the isolated kephalin — and sphingomyelin fractions. However, also these two phospholipids exerted a protective action against the lysolecithin-induced hemolysis. Thus approximately 15 μg P of the kephalin fraction and 40 μg P of the sphingomyelin fraction (material B Fig. 2) was needed to abolish the hemolysis due to 3 μg lysolecithin P. The amounts of these two lipids relative to lysolecithin were thus above that present in normal human serum, and their hemolysis inhibitory action can not explain why the total phospholipid fraction did not hemolyse red blood cells.

Hemolysis experiments were also carried out in a similar manner using the synthetic L-α-dipalmitoyl-lecithin. In contrast to the serum lecithin fraction, this synthetic lecithin did not show any marked inhibitory action in the test system used here. 3 μg lysolecithin P gave complete hemolysis when incubated with 30 μg synthetic-lecithin P and even with 80 μg synthetic lecithin P an almost complete (+++) hemolysis was observed.

DISCUSSION

The presence of lysolecithin as a normal lipid constituent was first suggested by Bergenheim & Fåhraeus in 1936. They isolated from serum a fraction that was soluble in water, ether insoluble, contained phosphorus and was strongly hemolytic. This fraction was later studied by Singer (1940, 1941) and by Singer & Dameshek (1941) in different hemolytic anemias, however witho being able to reach any conclusions as to physiological or clinical importance. By cently developed silicic acid column and pa chromatographic methods, the presence lysolecithin in lipid extracts of normal hum sera has been confirmed (Phillips 19 Gjone *et al.* 1959, Marinetti, Albrecht, F & Stotz 1959). At present it seems m likely that it is a true serum lipid compon and does not represent an artefact caused extraction or chromatography.

The present experiments demonstrate t the well known hemolytic activity of ly lecithin is not exerted by total serum-ph pholipid fractions containing lysolecithin amounts that in the isolated state would h given a very rapid and marked hemol *in vitro*. This has been found to be due p dominantly to an inhibiting action of serum lecithin on the hemolytic effect lysolecithin. If the same principle prevails serum, lysolecithin in the quantities so reported will not, due to the presence serum lecithin, exert any hemolytic act *in vivo*. Only marked alteration in the ative amounts of these two phospholipids serum would be expected to result in he lysis. Other inhibitory mechanisms may be present *in vivo, e.g.* by specific lipopro bondings. Kephalins and sphingomy exert a protective action but only in n physiologival quantities. Other serum li may possibly also have a similar action.

Rather surprising was the finding synthetic L-α-dipalmitoyl-lecithin lacked antihemolytic property of serum lecithin. possibility exists that some of the specifi of the inhibitory action is to be found in fatty acid portion of the lecithins. Howe

ifferent solubility properties in the test ystem used may also explain the discrepncy between the lecithins.

The present investigation shows that the arked hemolytic activity of isolated lysoecithin should not represent any objection the belief that lysolecithin is a normal lipid onstituent of human serum.

SUMMARY

1) The phospholipids of lipid extracts of ormal human serum were separated by eans of silicic acid column chromatography nto kephalins, lecithin, sphingomyelin and ysolecithin. Silicic acid impregnated paper hromatography was used for their identiication.

2) The isolated lysolecithin fraction exerted a marked hemolytic action on red blood ells. This action was not present in the otal serum phospholipid fraction.

3) The serum lecithin was found to ossess an inhibitory action on the hemolytic ctivity of lysolecithin. Relative values, ompared to those of normal human serum, re given.

REFERENCES

Bergenhem, B. & Fåhraeus, R.: Über spontane Hämolysinbildung im Blut unter besonderer Berücksichtigung der Physiologie der Milz. Ztschr. f. d. ges. exper. Med. *97*, 555, 1936.

Chargaff, E., Levine, C. & Green, C.: Techniques for the demonstration by chromatography of nitrogenous lipide constituents, sulfurcontaining amino acids, and reducing sugars. J. Biol. Chem. *175*, 67, 1948.

Gjone, E., Berry, J. F. & Turner, D. A.: The isolation and identification of lysolecithin from lipid extracts and normal human serum. J. Lipid Research *1*, 66, 1959.

Hirsch, J. & Ahrens, E. H. Jr.: The separation of complex lipide mixtures by the use of silicic acid chromatography. J. Biol. Chem. *233*, 311, 1958.

King, E. J.: The colorimetric determination of phosphorus. Biochem. J. *26*, 292, 1932.

Lea, C. H., Rhodes, D. N. & Stoll, R. D.: Phospholipids. On the chromatographic separation of glycerophospholipids. Biochem. J. *60*, 353, 1955.

Marinetti, G. V., Albrecht, M., Ford, T. & Stotz, E.: Analysis of human plasma phosphatides by paper chromatography. Biochim. Biophys. Acta. *36*, 4, 1959.

Phillips, G. B.: The isolation of lysolecithin from human serum. Proc. Nat. Acad. Sc. *43*, 566, 1957.

Singer, K.: The lysolecithin fragility test. Am. J. M. Sc. *199*, 466, 1940.

— Lysolecithin and hemolytic anemia. The significance of lysolecithin production in the differentiation of circulating and stagnant blood. J. Clin. Investigation *20*, 153, 1941.

— & Dameshek, W.: Symptomatic hemolytic anemia. Ann. Int. Med. *15*, 544, 1941.

Scandinav. J. Clin. & Lab. Investigation 374—376, 13, 1961.

QUANTITATIVE DETERMINATION OF URINARY GLUCOSE USING GLUCOSE OXIDASE AND PEROXIDASE

By L. KRAMER JAKOBSEN

From the Central Laboratory of Aalborg County Hospital, Denmark.

(Received for publication December 12, 1960)

Quantitative determination of glucose in urine can be carried out by the glucose oxidase method indicated by Keston (1956) and Teller (1956) without modification, although some authors (Huggett & Nixon 1957, Kreutzer & Bosman 1960, Saifer & Gerstenfeld 1958) claim that ascorbic acid and uric acid may interfere with color development.

METHOD

Reagents

a) Buffered enzyme-dye solution (GOD-POD reagent), *e.g.* TCM-II from C. F. Boehringer.

b) perchloric acid, 3.3 per cent (w/v). 28.5 ml acidum perchloricum Merck 1.67 is diluted with water to 1000 ml in a volumetric flask.

c) Standard solution of glucose. 10 mg ANALAR D-glucose is dissolved in 100 ml 3.3 per cent perchloric acid.

Procedure

Urine samples. The urine is diluted 1:100 with water in a volumetric flask. 200 µl of this dilution is pipetted into test tubes and 5 ml GOD-POD reagent added.

Standard. 5 ml GOD-POD reagent is added to 200 µl of the standard solution of glucose.

Blank. 5 ml GOD-POD reagent is added to 200 µl perchloric acid.

After 40 minutes at room temperature the optical densities (E) are read at 436 nm.

Calculation.

$$\frac{\text{Esample} - \text{Eblank}}{\text{Estandard} - \text{Eblank}} = \text{g glucose per 100 ml.}$$

COMMENTS

Ascorbic acid. Normally about 25 mg ascorbic acid is excreted per 24 hours. The color development of 10 mg/100 ml solutions of glucose in water with varying amounts of ascorbic acid was investigated. The results which are shown in Fig. A indicate that a urine specimen which after dilution contains 25 µg ascorbic acid per 100 ml (corresponding to 25 mg ascorbic acid per 1000 ml undiluted urine) gives no significant decrease of the optical densities.

Uric acid. Normally about 300 to 1000 mg uric acid is excreted per 24 hours. The color development of 10 mg/100 ml solutions of glucose in water with varying amounts of sodium urate was investigated. The results which are shown in curve B indicate that a urine specimen which after dilution contains 1 mg sodium urate per 100 ml (corresponding to 1000 mg sodium urate per 1000

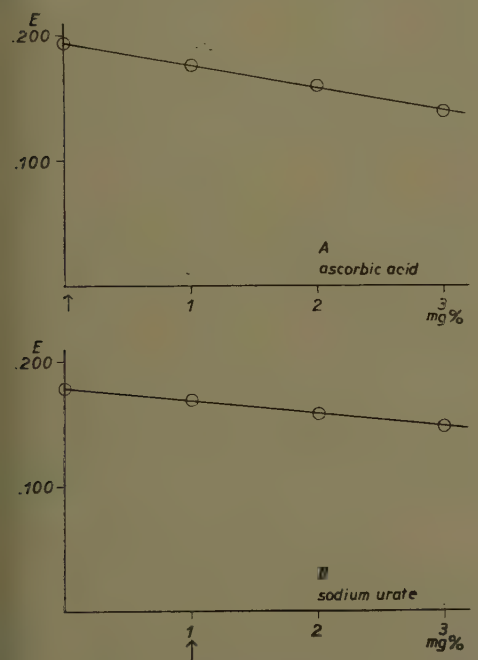
ıdiluted urine) gives a decrease of the op-
cal densities of about 0.007, which for clin-
al purposes is unimportant.

ecovery determinations.

According to Table I, good recoveries
ere obtained after addition of varying
nounts of glucose to urine.

recaution.

O'Gorman, Griffiths & Bloxam (1960)
ave shown that large doses of ascorbic acid
lministered a few hours before miction of
rine used for detection of slight glucosuria
an inhibit the enzymatic reaction. The ex-



ig. 1. Optical densities obtained with 10 mg/100
l solutions of glucose containing varying amounts
ascorbic acid and sodium urate. The arrows
dicate the concentrations of ascorbic acid and
odium urate which correspond to urines contain-
g 25 mg/l ascorbic acid and 1000 mg/l sodium
urate before dilution.

Table I. *Recovery determinations after addition of varying amounts of glucose to urine.*

Glucose added %	Glucose found %
0.0	0.0
0.5	0.5
1.0	1.0
1.5	1.5
2.0	2.0
2.5	2.6
3.0	3.0
3.5	3.6
4.0	4.1
4.5	4.6
5.0	5.1

cretion of uric acid in some leukemic cases, and during X-ray treatment, may exceed the above mentioned values, but under normal conditions the excretion of uric acid does not vary much.

Conclusion.

According to the present investigation, qualitative and quantitative determination of glucosuria can be carried out with great accuracy by the glucose oxidase method if intake of large amounts of ascorbic acid is avoided. It is convenient to run the urine analyses together with the blood glucose determinations (Kramer Jakobsen 1960, Raabo & Terkildsen 1960).

SUMMARY

The glucose oxidase method used for blood glucose determination can be used for urinary glucose determination without modification. Ascorbic acid and uric acid decrease the optical densities obtained, but for ordinary clinical purposes this is unimportant, when previous intake of large amounts of ascorbic acid is avoided.

REFERENCES

Huggett, A. St. G. & Nixon, D. A.: Use of glucose oxidase, peroxidase and o-dianisidine in determination of blood and urinary glucose. Lancet *II,* 368, 1957.

Jakobsen, L. Kramer: Quantitative determination of blood glucose using glucose oxidase and peroxidase. Scandinav. J. Clin. & Lab. Investigation *12,* 76, 1960.

Keston, A. S.: Specific colorimetric enzymatic analytical reagents for glucose. Abstr. Am. Chem. Soc. 129th meeting, 31 C, 1956.

Kreutzer, H. H. & Bosman, M.: Enzymatische bepaling van het bloedsuikergehalte. Nede tijdschr. v. geneesk. *104,* 1379, 1960.

O'Gorman, P., Griffiths, P. D. & Bloxam, H. R Ascorbic acid inhibition of the glucose oxida test for glucosuria. Brit. M. J. *1,* 603, 1960.

Raabo, E. & Terkildsen, T. C.: On the enzymat determination of blood glucose. Scandinav. Clin. & Lab. Investigation *12,* 402, 1960.

Saifer, A. & Gerstenfeld, S.: The photometric m crodetermination of blood glucose with gluco oxidase. J. Lab. & Clin. Med. *51,* 448, 1958.

Teller, J. D.: Direct quantitative colorimetric d termination of serum or plasma glucose. Abs Am. Chem. Soc. 130th meeting, 69 C, 1956.

Scandinav. J. Clin. & Lab. Investigation 377—389, 13, 1961.

ESTIMATION OF THROMBOPLASTIN ACTIVATION IN PLASMA

By T. ASTRUP[1] and P. OLLENDORFF

From the Biological Institute, Carlsberg Foundation, Copenhagen, Denmark

(Received for publication December 6, 1960)

An estimation of the initial activation of blood coagulation is of interest for many reasons. This interest ranges from the study of mild hemophilioid disorders to investigations in the influence of diets on the clotting of blood in normal persons; from studies aimed at an elucidation of the mechanism of blood clotting to an estimation of hypercoagulability in patients. Several methods have been proposed for the estimation of the initial activation of blood coagulation, but so far none have been generally accepted.

Many problems, theoretical as well as practical, are involved in an investigation of the initial activation of blood clotting. After the process of blood coagulation has been divided into two different systems corresponding to the socalled extrinsic system and the intrinsic system, respectively, some simplification has been possible.

The spontaneous clotting of blood in a test tube is caused primarily by activation of the intrinsic system. Thus the spontaneous clotting time is normal in patients lacking Factor VII, Jürgens 1956, Voss & Waaler 1959. This is also the reason why the thrombin generation test is normal in Factor VII deficient patients (Hicks 1955, Ackroyd 1956).

Observations such as these are indicative of the significance of the plasma thromboplastin system in the initiation of normal blood coagulation.

The purpose of the present paper is to investigate the initial activation of plasma thromboplastin in order to be able to estimate the latent activity of the intrinsic system and to detect its variations within the normal physiological range.

1. SELECTION OF METHOD

Various methods have been recommended for investigation of the plasma thromboplastin system. The thromboplastin generation test (TGT) of Biggs & Douglas (1953) marked a great step forward, since it permitted the separate estimation of the two main components of the plasma thromboplastin system, namely AHF and the Christmas factor. However the method is based on the use of serum as one of the test ingredients. In normal serum initiating stages of the clotting process have already been completed. An identification of a serum factor with a factor in genuine plasma requires therefore rigid proof since an effect caused by serum might be different in nature from an apparently similar effect caused by a plasma factor (Astrup 1959). Miale & Garrett (1957) arrived at

[1] Present adress: James F. Mitchell Foundation, Institute for Medical Research, Washington Clinic, 5401 Western Ave. NW., Washington 15, D.C.

the conclusion that "the identity of "plasma factors" and "serum factors" may be more apparent than real".

MacMillan & Ritter (1958) avoided the use of serum as a source of Christmas factor in the test by substituting with diluted plasma in order to make the test more sensitive to abnormalities in the earlier stages of thromboplastin formation.

Another method which avoids serum and is recommended in the study of the activation of plasma thromboplastin is the partial thromboplastin time test (PTT test or cephalin clotting time test) used extensively by Brinkhous and his associates in their work on classical hemophilia (AHF-deficiency), (Langdell, Wagner & Brinkhous, 1953). Rodman, Barrow & Graham (1958) concluded that the PTT-test is more sensitive than the thromboplastin generation test. However, since the PTT method is a one stage method it is not possible to follow the thromboplastin formation with time.

Another method avoiding addition of serum is the "thromboplastin screening test" of Hicks & Pitney, 1957. In this method diluted, whole plasma is recalcified in the presence of a platelet substitute. In several cases of mild defects, where the usual thromboplastin generation test gave normal results, the screening test was reported abnormal. Apparently this test is more sensitive than the original thromboplastin generation test. This observation was confirmed by Biggs, Sharp, Margolis, Hardisty, Stewart & Davidson, 1958, who used an improved "thromboplastin screening test" in which plasma was obtained with silicone technique.

Common to all the methods so far mentioned is an acceleration of the velocity of thromboplastin formation by addition of platelets or platelet substitutes. Hence, the methods are more appropriate in an estimation of total thromboplastic activity than in an estimation of the initial activation of thromboplastin formation. Addition of washed platelets or a platelet substitute introduces an artefact which could easily conceal defects in the plasma sample caused by a defective platelet system. Evidently, in order to obtain information about the initial steps in thromboplastin formation under physiological conditions, it is essential that the plasma sample remain as undisturbed as possible, which means that it should also contain its own untreated platelets.

A method using a genuine sample of blood is the thrombin generation test described for whole human blood by Macfarlane & Biggs (1953) and for citrated plasma by Pitney & Dacie (1953). Using a substitution principle the thrombin generation test can be used in the demonstration of defects in the plasma thromboplastin system (Sjølin, 1956 a, 1957 b, 1960, Astrup & Sjølin, 1957). The thrombin generation test is more sensitive than the thromboplastin generation test of Biggs & Douglas in demonstrating mild defects in the plasma thromboplastin system (Sjølin, 1957 a, 1958 a, 1960, Ollendorff, 1959).

However, in mild deficiencies also the thrombin generation test often fails for which reason Ollendorff (1960 a) tried to improve the method and make it more accurate.

The method gives qualitative information, and it expresses the total activity of the plasma thromboplastin system, though the lag period in the test and also the steepness of the curves can give some idea about the velocity of thromboplastin generation (Dybkær & Geill, 1959).

A method estimating preferably the total concentration or total activity of any factors or intermediate products cannot be expected to give a true picture of variations in the velocity of the initial reactions.

In order to accomplish this, strict adherence to silicone technique is essential. Douglas & Mai (1958) have described a test which they call the "thrombin-thromboplastin generation test". In principle this test is very similar to the previously mentioned "thromboplastin screening test", but no platelets or platelet substitute are added to the test sample. The principle used by Douglas & Mai (1958) is, therefore, much better suited as an indicator of the early development of activity during the process of clotting. Unfortunately this advantage is again lost, since the authors use ordinary, uncoated glassware, so that part of the initial activation has already taken place during the preparation of plasma samples and of the substrate plasma. This is evident from the short clotting times obtained in their experiments. Furthermore, plasma samples which have been stored

at 20° C, are used. This treatment produces partial activation even in silicone coated glass tubes, which in certain mild cases of hemophilioid disorders is sufficient to overcome completely the clotting defect in the genuine plasma sample (Sjølin, 1956 b, 1957 a, 1958 a, 1960, Biggs *et al.*, 1958).

In the following we describe experiments aimed at the development of a method for the estimation of the initial activation of plasma thromboplastin. The method, as finally adopted, is described, and some preliminary results obtained on a limited material of normal persons and patients are reported in order to demonstrate the applicability of the method. The principle underlying the method is the same as that applied by Douglas & Mair (1958), but the technique has been modified, so that a high sensitivity of the method has been obtained. Since the method has been designed for the study of the initial activating steps, with little emphasis on the total activity generated, we refer to the method as the *thromboplastin activation test* (TAT). The method must not be confused with the "thromboplastinogen activitiy time" of Quick, Hussey & Stapp (1952), which is a modified partial thromboplastin time test, using the one-stage principle.

2. MATERIALS AND METHODS

Strict silicone technique is adhered to during all manipulations. An exception is the stainless steel cannulas, which are used uncoated since it was felt that a more smooth surface could be maintained in these when not exposed to the hydrochloric acid released during silicone treatment. Handling and cleaning of glassware has been described in detail in another paper (Ollendorff, 1960 a). It is of great significance that all pipettes are placed in 0.5 n NaOH, since adsorption of active agents can give erroneous results in subsequent estimations. Dichromate-sulfuric acid can be used instead of 0.5 n NaOH but requires longer time.

Substrate plasma (platelet poor): Human blood (9 vol. to 1 vol. 3.8 per cent trisodium citrate) was, after clean venepuncture, bled directly into a silicone coated centrifuge tube discarding the first few ml. After centrifugation in this tube for 30 min. at 3500 r.p.m. in a refrigerated centrifuge, the platelet-poor plasma was carefully separated with a silicone-coated pipette and kept in icewater in a silicone-coated flask.

In some experiments a "high spun" plasma prepared by high speed centrifugation in a refrigerated centrifuge (18 000 r.p.m. for 30 min.) was used as substrate plasma. However, when the normal platelet-poor substrate plasma was of good quality, no improvement was obtained by further high speed centrifugation. The applicability of the normal substrate plasma depends upon the amount of material from disintegrated platelets present in the original citrated plasma, *cfr.* Ollendorff (1960 c), and this could be controlled by estimating the recalcification time of the substrate plasma, see below.

Plasma sample (platelet-rich): The blood sample to be tested was obtained as the substrate plasma. The platelet-rich plasma was separated by centrifugation at 1 500 r.p.m. for not more than 10 min. in a refrigerated centrifuge. The sample was kept in icewater during the experiment.

Platelets were counted in the plasma sample in a 0.05 mm deep Bürker-Türk counting chamber by the direct method.

Calcium chloride for recalcification was used in the following concentrations: 0.025 M CaCl in water, and a 0.0083 M solution prepared by diluting the 0.025 M solution with two volumes of buffer.

Saline-barbital buffer: 0.05 M diethylbarbital buffer; pH 7.75 containing 0.1 M NaCl. Total ionic strength 0.15.

Thromboplastin activation test (TAT): Based upon the results obtained in a number of experiments (of which some will be reported below) the following conditions were found to be optimal.

Diluted substrate plasma was prepared by diluting with 2 vol. of buffer and the mixture was kept in icewater during the experiments in a silicone-coated glass container.

Technique of thromboplastin activation test: Of the *diluted substrate plasma* aliquots of 0.100 ml were placed by means of an uncoated pipette in each of a series of 15 uncoated tubes (80 mm × 9—10 mm) and the tubes were placed in a waterbath at 37° C.

In a larger uncoated test tube (95—100 mm ×

12—14 mm) 2.00 ml buffer was mixed with 0.250 ml 0.025 M $CaCl_2$ and the tube placed in the water bath. After reaching the temperature of the bath, 0.250 ml of the *platelet-rich plasma sample* was added (with an uncoated pipette) and a stop watch immediately started.

At each following whole minute a subsample of 0.100 ml of this incubation mixture was removed by a micropipette and consecutively placed in one of the above mentioned 15 test tubes containing the substrate plasma. At the same time as the subsample was introduced in a tube, 0.100 ml of the 0.0083 m $CaCl_2$ solution was added for recalcification. The clotting time was then estimated. Because of the long recalcification time of the substrate plasma the recalcification can — if technically easier — be performed with an automatic pipette a few seconds before the subsample is added.

The clotting time of the incubation mixture was also recorded. The clot formed was pressed against the wall of the test tube by means of two wooden applicator sticks in order to squeeze out the solution.

The clotting times of each of the 15 test tubes were read (t_1 to t_{15}). The results were plotted with the incubation time (in minutes) as abscissa and the logarithm of the clotting times (in seconds) as ordinate, preferably on semilogarithmic paper. The curve thus obtained is the activation curve.

Estimation of t_o.

In order to obtain a more accurate estimation of the zero point (t_0) of the activation curve, which point represents the activity present in the genuine, untreated plasma sample, t_0 was obtained as follows:

In each of 3 uncoated test tubes 0.100 ml diluted substrate plasma was placed, and the tubes placed in a water bath at 37° C.

In a larger, silicone-coated tube, 2.25 ml buffer and 0.250 ml of the plasma sample was mixed and placed in the water bath. At short intervals 0.100 ml of this mixture was transferred to one of the 3 tubes containing the substrate plasma at the same time as 0.100 ml 0.0125 M $CaCl_2$ (= 0.025 M $CaCl_2$ diluted with one volume of buffer) was blown into the tube. A stop watch was started for each tube and the clotting time estimated. The average of the three values wos considered as an expression of t_0.

Recalcification time of substrate plasma.

In order to determine whether the platelet-poo substrate plasma was satisfactory for the test, it recalcification time was estimated as follows:

In an uncoated test tube was placed 0.100 ml o the diluted substrate plasma followed by 0.100 m buffer and 0.100 ml 0.0083 M $CaCl_2$ (0.025 M $CaCl_2$ diluted with 2 vol. of buffer). The clottin time was estimated in three consecutive tests an the average calculated. If, in this test, the recalci fication time was lower than 4 minutes, the sub strate plasma was unsuitable and had to be re placed by another preparation.

3. INVESTIGATION ON THE METHOD

A. Substrate plasma and plasma sample.

In preliminary experiments various dilutions o substrate plasma and plasma samples were trie Finally adopted were those concentrations whic gave the best range of readings as well as the be reproducibility. Of great importance is the qualit of the platelet-poor substrate plasma. When th clotting time of the latter, estimated as describe above, was above 4 minutes, no further significa improvement was produced by high-speed centr fugation. When the substrate plasma was of inferi quality, because it contained disintegrated platel material, it could usually not be sufficiently im proved by high speed centrifugation. Hence, gre care is required in preparing the substrate plasm In some experiments the human substrate plasm was exchanged with a platelet poor, oxalated bovi plasma with good results.

The final dilution of the platelet-rich plasm sample in the incubation mixture was selected 1 in 10. Hicks & Pitney (1957), in their thromb plastin screening test, used an incubation mixtu containing the plasma sample diluted 1 in 30. Big *et al.* (1958) used 1 in 60 in their incubation mi ture. Douglas & Mair (1958), in their thrombi thromboplastin generation test, used a final dilutio of 1 in 6. The substrate plasma used by all the

ors was undiluted. We found it advantageous se a diluted substrate plasma since the clotting s are then slightly prolonged, so that minor ations in activity of the incubated plasma ple could be more accurately determined.

The thrombin effect.

Thrombin as well as thromboplastin is formed he incubation mixture. Since we are primarily rested in the activation of plasma thromboplas- any formation of thrombin in the plasma sample sents a complication of the method. However, n undisturbed plasma is used, thrombin forma- is unavoidable. It therefore became of signif- ce to investigate how much the presence of mbin in the sample interfered with the estima- of thromboplastin activation on the substrate ma.

From an incubation mixture prepared as de- bed above, aliquots (0.100 ml) were removed ne minte intervals and added to tubes contain- 0.200 ml of a 0.075 per cent solution in buffer ovine prothrombin-free fibrinogen (prepared as cribed by Astrup & Müllertz, 1952). The clott- times were recorded. In this experiment clott- is caused by the thrombin formed in the in- ation mixture alone. The results were compared h those obtained in the usual procedure using ted plasma as substrate and carried out on the e plasma sample. The results of such an exper- nt are presented in Fig. 1. The sample of normal sma used in this experiment contained 384.000 telets per mm^2. It is seen that during the first o 6 mins. the activity in the plasma sample is sed chiefly by the thromboplastin formed. Later effect is due primarily to thrombin.

Variations in substrate plasma.

In order to follow variations in plasma samples h time, either during storage or as obtained m individuals at different times, it is important know how much the properties of different sub- ate plasma preparations deviate from each other. obtain some information on this problem the sitivity of various plasma substrates to thrombin s studied. A purified, lyophilized preparation of ine thrombin was used (kindly provided by the

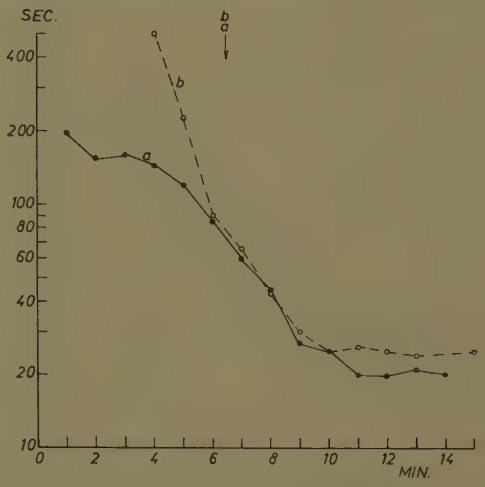


Fig. 1. *Role of thrombin effect.*

Curve a: Thromboplastin activation test. *Curve b:* Same incubation mixture estimated on bovine fibrinogen. *Abscissa:* Incubation time in minutes. *Ordinate:* Logarithm of clotting time in seconds. Arrows marked a and b indicate recalcification time of the respective incubation mixtures.

Leo Pharmaceutical Comp., Copenhagen). It was dissolved in buffer in an appropriate concentration and serial dilutions were prepared. With these dilutions the clotting of the substrate plasma was investigated by addition of 0.100 ml of the thrombin solutions and of 0.100 ml of 0.0083 M $CaCl_2$ to 0.100 ml diluted substrate plasma (1 in 3). Results of such experiments are presented in Fig. 2. It is seen that the deviations between 12 different preparations of substrate plasma obtained during 4 weeks and tested at various days with thrombin are small. It is therefore assumed that curves obtained on different days and with different batches of substrate plasma can be compared. The method has been in use now for more than two years and the results obtained during this period have verified this assumption. It would have been advisable also to investigate the sensitivity to dilutions of plasma thromboplastin, but since stable preparations of this are not available such an investigation could not yield definite results.

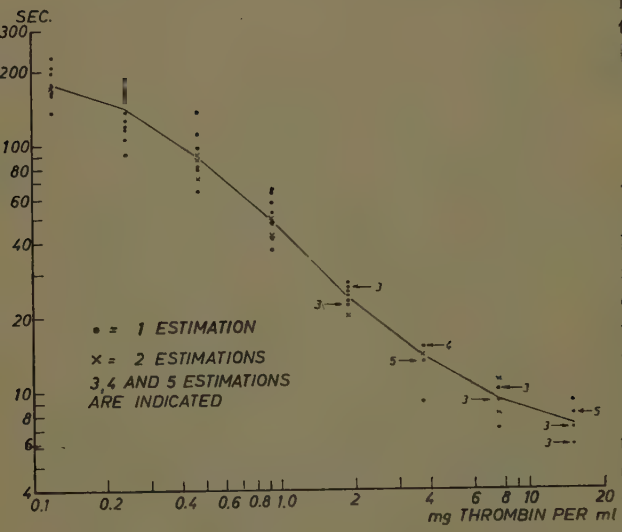


Fig. 2. *Substrate plasma variations.*

Abscissa: Conc. of thrombin in mg of thrombin preparation per 10 ml buffer. *Ordinate:* Clotting time in secs. Both axes logarithmic. Results obtained in 12 different samples of substrate plasma are plotted. Full line is average curve.

D. Reproducibility of estimations.

In order to obtain information about the reproducibility of the estimations the activation curve was estimated on one and the same sample of plasma in 2 separate experiments on one day and in 7 separate experiments the following day. The results appear in Fig. 3. It is seen that there is very little deviation in the beginning and the end of the curves. The total activity obtained is nearly the same in all experiments. There are slight deviations in the intermediate range, where the acceleration of activation occurs. The deviation is not seen in the slopes of the curves, but as variations in the lag period at the beginning of the nearly linear part of the accelerating section of the different curves. This probably means that there are no significant variations in the accelerating stage, but that there is a slight influence on the lag period. This could be caused by minor variations the technical handling of each separate experime which is difficult to avoid.

4. APPLICABILITY OF THE METHOI

A. Variations in single individuals.

As a prorequisite for the method in studies human beings it is necessary to know the variat in estimations carried out on single individu during a prolonged period of time. It was fo that some variation in the activation rate occ in one single individual with time. An example shown in Fig. 4 which shows 7 estimations o normal person (female) during 40 days. Appare there is a slight physiological variation in the acti tion rate. All blood samples were obtained in morning before eating.

B. Variation between normal individuals.

Two groups of normal persons were investiga The first group included 39 persons (11 males 28 females) from 18 to 56 years old and consi of hospital personal (doctors, nurses, technici

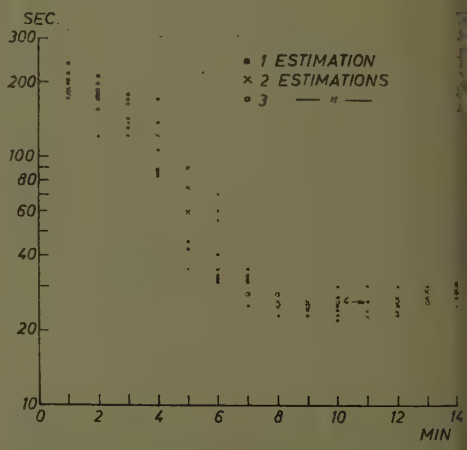


Fig. 3. *Reproducibility of estimations.*

9 separate thromboplastin activation tests perfo ed on the same sample of plasma on two consecu daws and estimated on the same substrate pla *Abscissa:* Incubation time in minutes. *Ordi* Logarithm of clotting time in secs.

. The results of these estimations, which were ormed in the period from Dec. 1958 to April ', are presented in Fig. 5.

: order to characterize each curve the follow- values were read:

t_1 = clotting time produced by the subsample obtained one minute after recalcification of the incubation mixture.

$_{ax}$ = time (in minutes) elapsed before the shortest clotting time was obtained on the curve.

$_{ax}$ = clotting time in seconds at T_{max}.

alc. = clotting time of the recalcified incubation mixture.

hese values are presented in Table I. There ome individual variation.

s mentioned above, the thromboplastin activity ne genuine plasma sample at zero time is rep- nted by t_0. Since t_0 has to be obtained in a rate experiment (as described) while t_1 can be ined from the regular test, it was investigated ther t_1 deviated so little from t_0 that it could be instead of t_0 as a measure of the latent clotting ity of the original plasma sample. In 16 of the nal persons of the group investigated above, t_0 estimated in separate experiments as described,

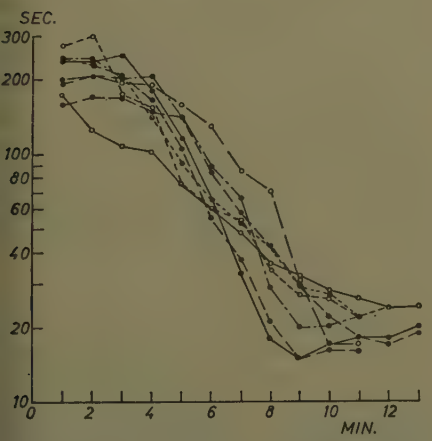


Fig. 4. *Estimations on a single individual.* rves obtained in one person (female, normal) ng a period of 40 days. *Abscissa* and *Ordinate* as described.

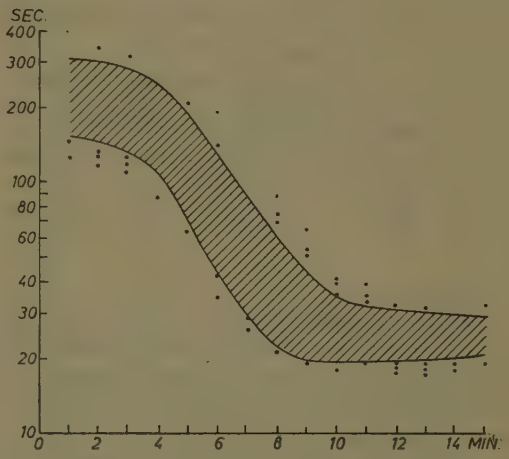


Fig. 5. *Range of estimations performed in 39 normal persons. Abscissa* and *Ordinate* as described. The hatched area cover 90 per cent of the range. The dotted points outside this area represent the remaining 10 per cent of the values.

each estimation being the average of 3 readings. Table II describes the results. It is seen that there is very little difference between t_0 and t_1 so that the latter can be used instead of t_0, which greatly simplifies the estimations.

Another group of normal persons was investigated

Table I. *Values obtained in 39 nomal persons.*

	t_1 in sec.	T_{max} in min	t_{max} in sec.	Recalc. in sec.
Average	213.3	11.3	22.9	412.6
Highest value	405	15	30	540
Lowest value	125	8	13	320

t_1 = clotting time in secs. of substrate plasma efter addition of a subsample transferred from incubation mixture after one minute. T_{max} = time (in min.) elapsed until shorthest clotting time is reachd. t_{max} = clotting time at T_{max}. Recalc. = clotting time of incubation mixture.

Table II. *Values of t_0 and t_1, obtained from 16 normal persons.*

	Average in sec.	Highest value	Lowest value
t_0	216	278	154
t_1	229	340	183

in the period from Oct. 1959 to Feb. 1960. This group consisted of blood donors and included males and females. The results obtained are presented in Fig. 6. It is seen that this group differs slightly from the previously mentioned normal group, since on an average there is an increased steepness of the curves indicating more rapid acceleration of thromboplastin formation. The reason for this difference could be that the latter group, consisting of donors, represents individual blood samples obtained at different times during the day. Blood samples obtained in the morning and during the first hours of the afternoon were tested on the same day, whereas samples obtained in the evening or at night had to be stored in icewater until they could be tested the following morning. The res presented in Fig. 6 are therefore more representa than the material in Fig. 5 for samples obtaine random periods during the day, such as is often case in clinical investigations (Ollendorff, 1960

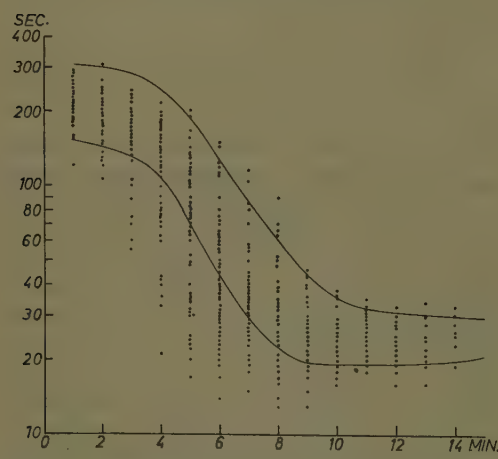


Fig. 6. *Results obtained on 79 normal persons (blood donors).* The two lines mark the area of the normal range obtained in the previous group in Fig. 5. Several of the spots represent more than one estimation.

C. Results obtained on patients with class hemophilia or Christmas disease.

The applicability of the test in the demonstra of AHF-deficiency or Christmas factor deficie was investigated. The results are presented in 7 and Fig. 8. The preparation of the reagents u in these investigations is described elsewhere (S lin, 1956 a, 1957 a, b, 1960).

It is seen that plasma from a patient lack antihemophilic factor (Fig. 7) shows almost formation of thromboplastin (curve a). Addition adsorbed, oxalated bovine plasma to the incuba mixture brought the activation curve in the nor range (or even slightly increased) (curve Addition of normal, human serum (curve c) readsorbed, heat-treated, normal serum (curve had only little effect on the activation.

Simiarly in Fig. 8 it is seen that in plasma f a patient deficient in Christmas factor there is slight thromboplastin activation (curve a). Addi of normal serum produced normal activa (curve b), while addition of readsorbed bo plasma had only slight effect (curve c). The clot times (recalc.) of the incubation mixtures marked on the curves for comparison.

It is apparent from these results that the met can be used in the diagnosis of hemophilioid orders using the same principle of substitution wh proved so valuable in the application of the throm generation test (Sjølin 1956 a, 1957 b, 1960, As & Sjølin, 1957).

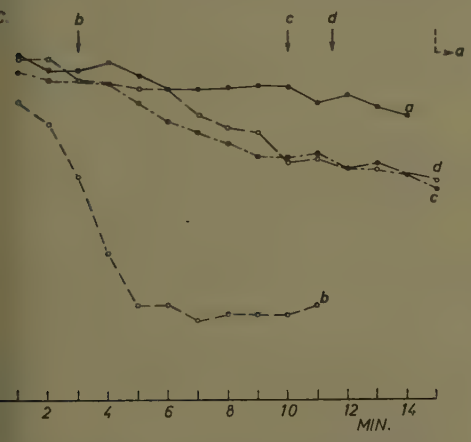
D. Presence of tissue thromboplastin.

In order to be able to evaluate estimations tained on patients in whom hypercoagulability the blood might be expected, investigations w performed on the effect on the test of additio tissue thromboplastin. Such experiments m simulate the composition of blood in patients

us forms of tissue injury producing release of thromboplastin into the blood stream. Some s are presented in Fig. 9. It is seen that even concentrations of tissue thromboplastin in the ation mixture greatly accelerate the clotting ss. Since tissue thromboplastin acts directly e prothrombin system without the intermediate ation of plasma thromboplastin the activation e clotting process observed after addition of thromboplastin is due to the rapid direct ation of thrombin.

lass activation of plasma thromboplastin.

some experiments the effect of glass activation studied in order to simulate an increased activ- the plasma thromboplastin system in a manner would not introduce foreign, active material



7. Application of the thromboplastin activation n plasma from a patient with classical hemophilia.

e a: Genuine plasma sample from patient. *e b:* Incubation mixture contained 0.05 ml O_4-adsorbed bovine plasma (= 20 per cent of .25 ml patient plasma present). *Curve c:* In- ion mixture containing 0.05 ml normal, human n. *Curve d:* Incubation mixture contained 0.05 adsorbed, heat-treated human serum. Arrows and d indicate recalcification times of the ctive mixtures. Recalcification time of mixture a was longer than 15 mins.

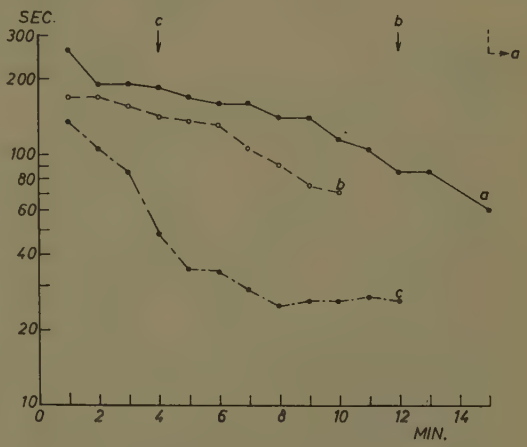


Fig. 8. *Thromboplastin activation test in plasma from a patient with Christmas disease.*

Curve a: Genuine plasma sample from the patient. Platelet count: 386 000. *Curve b: Incubation mixture contained 20 per cent adsorbed bovine plasma. Curve c: Incubation mixture contained 5 per cent normal human serum.* The respective clotting times of the incubation mixtures are indicated with arrows. (Mixture a: longer than 15 mins.)

in the test system. Fig. 10 shows an experiment in which citrated rabbit plasma was used in the incubation mixture. The substrate plasma was of human origin as usual. Curve a shows the thromboplastin activation in the untreated rabbit plasma. It is very similar to the curve obtained in normal human plasma. Curve b shows the accelerated activation in the rabbit plasma after it had been treated for 30 mins. at room temperature with 200 mg glass powder per ml plasma. Curve c shows the accelerating effect of normal human serum on the thromboplastin activation. The incubation mixture contained 0.05 ml serum per 0.25 ml rabbit plasma. It is seen that the accelerating effect of glass treatment is nearly as high as that produced by serum addition. This effect is also apparent from the recalcification times of the incubation mixtures.

The similarity of the curves in Fig. 10 with those obtained by addition of tissue thromboplastin in Fig. 9 made it necessary to investigate the formation of thrombin during the accelerated

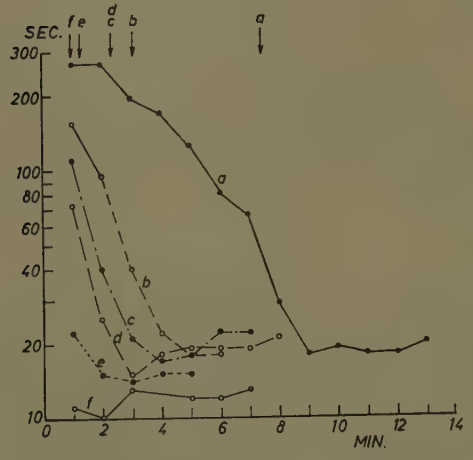


Fig. 9. *Thromboplastin activation test in the presence of tissue thromboplastin.*

Curve a: Normal human plasma alone. Platelet count: 735 000. *Curves b—g:* Incubation mixture contained human brain thromboplastin (Owren) in the following concentrations per 0.25 ml of plasma: b: 0.5 µl; c: 5 µl; d: 10 µl; e: 50 µl; f: 100 µl. The respective clotting times of the incubation mixtures are indicated with arrows for comparison.

formation of plasma thromboplastin, since the curves in Fig. 9 were caused by direct activation of the prothrombin system, whereas the curves obtained in Fig. 12 were due to an activation first of the plasma thromboplastin system. Such an experiment is presented in Fig. 11. It is seen that acceleration of thromboplastin activation results in an acceleration of thrombin formation, which nearly follows the thromboplastin activation curve as soon as the shorter clotting times are reached. This result is again an indicatian that it is the initial part of the curve which is of interest more than the part representing total or nearly total activation. This has already been mentioned in a previous section. The latter part is identical with a thrombin generation test performed in a higher dilution of plasma than usual.

DISCUSSION

An estimation of the initial activation plasma thromboplastin in genuine plas samples presents great difficulties. The gr est obstacle is the simultaneous formatio thrombin, since no method is known wh can prevent the formation of thrombin can make thrombin inactive without in fering with the formation or estimatio plasma thromboplastin. However infor tion about the activity of the latent plas thromboplastin system is significant many attempts have been made to de suitable methods. All of these have to c promise on various points. An indirect e mation of plasma thromboplastin in genu untreated plasma can be obtained by thrombin generation test. However, the tial phases of thromboplastin activation

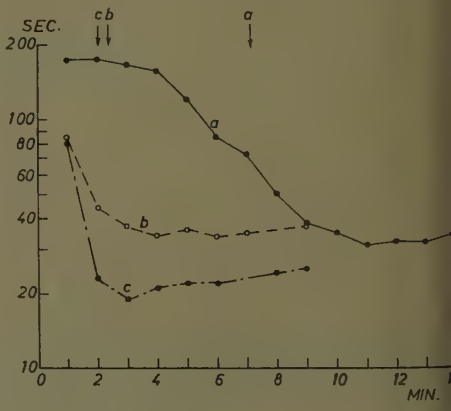


Fig. 10. *Thromboplastin atcivation in rabbit pl and accelerating effect of glass treatment serum addition.*

Curve a: Normal untreated citrated rabbit pla *Curve b:* Rabbit plasma treated with 200 ml powder per ml before test. *Curve c:* Incub mixture containing 0.05 ml normal, human s per 0.25 ml normal rabbit plasma. Recalcific times of incubation mixtures indicated with ar for comparison.

er estimated in a method using the prin-
e of the "thrombin-thromboplastin gene-
on test" of Douglas & Mair (1958). The
ons for selecting this principle and for
king out the present method have been
ribed in the introductory section. It has
the purpose of this study to describe
background and technique of the new
nod as well as its application, usefulness
limitations. A similar procedure was
by Astrup & Fischer (1936) in their
attempt to obtain a quantitative esti-
on of the activation process in the clot-
of hen plasma. This plasma was used
use it is easy to prepare free from throm-
tes and is stable without addition of
coagulants, so that with this plasma it is
ible to approach physiological conditions.

n estimation method introducing as few
ical factors as possible is required if
ations in coagulability are to be accurat-
estimated and elucidated, such as those
ribed for mild hemophilioid defects in
ents by Sjølin (1958 b, 1960) and in
e blood by Ollendorff (1959). Here it
ie initial activation which is of interest.
activity obtained later is more an ex-
sion of the total activity obtainable and
ich does not reflect the initial activation
city. However, the period of time elapsed
maximal activation is of significance
is easily estimated by the present meth-
It is of less importance that at this high
ity the estimations are complicated by
additional activity of the thrombin form-
ince a rapid formation of thrombin is an
cation of an accelerated thromboplastin
ation. Such indirect estimations have
dy been used by application of the
mbin generation test (see above).

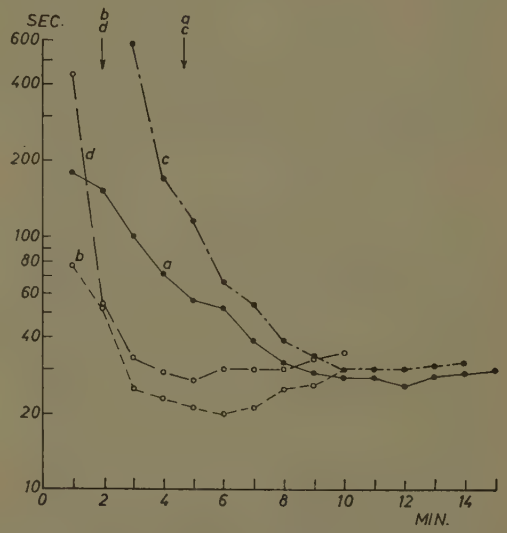


Fig. 11. *Thromboplastin activation and thrombin formation during acceleration of clotting.* *Curve a:* Thromboplastin activation test on normal human plasma. Platelet count: 384 000. *Curve b:* Thromboplastin activation test after glass treatment of plasma sample. *Curves c and d:* Same as a and b but thrombin formation followed by estimation on fibrinogen solution (bovine, 0.15 per cent). Ordinate in all instances expresses clotting time in secs.

The method described here has now been in routine use for more than two years. It has been found reliable and accurate in daily use and has given valuable information on the initial activation of coagulation in experimental work as well as in a variety of clinical conditions. The effect of platelets and of glass on the activation of plasma thromboplastin has been studied (Ollendorff, 1960 c). The clotting defect in Hagemann plasma was studied with the method (Ollendorff, 1961). The coagulability of blood in acute myocardial infarction was also studied (Ollendorff, 1960 d). The experience obtained in these, and in other studies to be published,

has demonstrated the applicability of the method, and we feel that it has advantages over other methods when an estimation of the initial activity of the clotting system in undisturbed blood is required.

SUMMARY

1. The conditions for an accurate estimation of the initial activation of the plasma thromboplastin system and the latent clotting activity in undisturbed blood are discussed.

2. An estimation method is described, which as far as possible satisfies these conditions.

3. The reliability of the method is demonstrated on blood samples from normal persons.

4. The application and limitations of the method are demonstrated in samples of blood from patients deficient in anthihemopilic factor or in Christmas factor, as well as in blood activated with small amounts of tissue thromboplastin or by glass treatment.

ACKNOWLEDGMENTS

This work was aided by a grant from the Josiah Macy Jr. Foundation, New York. One of us (P. O.) received a grant in aid from the "Købmand i Odense Johann og Hanne Weimann, f. Seedorff's legat". Thanks are due to Dr. Levin Nielsen, Frederiksberg Hospital, for permission to obtain blood samples from blood donors, and to Mrs. Aase Barrit who provided most of the other normal blood samples. The skillful technical assistance of Miss Lise Lolle and Miss Lis Kristiansen is acknowledged.

REFERENCES

Ackroyd, J. F.: The function of factor VII. Brit. J. Haemat. *2*, 397, 1956.

Astrup, T.: Plasma factors versus serum factors in blood coagulation. In: New Blood Clotting Factors. Transactions of the Montreux Conference, 1959, p. 186. Thromb. Diath. haemo Vol. 4, supplementum.

— Fischer, A.: Über den Reaktionsverlauf induzierten Gerinnungsvorganges im B plasma. Skandinav. Arch. f. Physiol. *74*, 1936.

— Müllertz, S.: The fibrin plate method estimating fibrinolytic activity. Arch. Bioch Biophys. *40*, 346, 1952.

— Sjølin, K.-E.: Defects in blood coagulat Dan. Med. Bull. *4*, 164, 1957.

Biggs, R. & Douglas, A. S.: The thrombopla generation test. J. Clin. Path. *6*, 23, 1953.

— Sharp, A. A., Margolis, J., Hardisty, R. Stewart, J. & Davidson, W. M.: Defects in early stages of blood coagulation: A repor four cases. Brit. J. Haemat. *4*, 174, 1958.

Douglas, A. S. & Mair, K.: The Christmas fa deficiency in cotmarin therapy. Clin. Sc. 445, 1958.

Dybkær, R. & Geill, T.: The thrombin genera test in plasma. Acta Haemat. *21*, 156, 1959

Hicks, N. D.: A coagulation disorder due t factor VII-like defect. M. J. Australia *2*, 1955.

— Pitney, W. R.: A rapid screening test for orders of thromboplastin generation. Brit Haemat. *3*, 227, 1957.

Jürgens, J.: Kongenitaler Faktor VII (SPC Mangel als Ursache einer hämophilie-art hämorrhagische Diathese. Acta Haemat. 181, 1956.

Langdell, R. D., Wagner, R. H. & Brinkhous M.: Effect of antihemophilic factor on stage clotting tests. J. Lab. & Clin. Med 637, 1953.

Macfarlane, R. G. & Biggs, R.: A thrombin ge tion test. J. Clin. Path. *6*, 3, 1953.

MacMillan, R. L. & Ritter, H.: A modificatio the thromboplastin generation test. Am. J. Sc. *235*, 437, 1958.

Miale, J. B. & Garrett, V. R.: Studies on thromboplastin generation test. III. The ef of dilution, storage and concentration of p lets. Am. J. Clin. Path. *27*, 701, 1957.

Ollendorff, P.: Defects in and variability o thromboplastic system in horse plasma. Thr Diath. haemorrh. *4*, 45, 1959.

Improvements in the thrombin generation test. Thromb. Diath. haemorrh. *4*, 244, 1960 a.

Platelet activity and activation of blood clotting by glass. Thromb. Diath. haemorrh. *4*, 410, 1960 c.

Coagulability of blood in acute myocardial infarction. Acta med. Scandinav. 169, 373, 1960 d.

Glass effect and platelet activity in Hageman plasma. Thromb. Diath. haemorrh. 1961 (in press).

ey, W. R. & Dacie, J. W.: A simple method of studying the generation of thrombin in recalcified plasma. J. Clin. Path. *6*, 9, 1953.

k, A. J., Hussey, C. V. & Stapp, W. F.: The effect of heating on the thromboplastic activity of rabbit brain extract. A new test for the diagnosis of hemophilia. J. Lab. & Clin. Med. *39*, 142, 1952.

man, N. F., Barrow, E. M. & Graham, J. B.: Diagnosis and control of the hemophilioid states with the partial thromboplastin time (PTT) test. Am. J. Clin. Path. *29*, 525, 1958.

in, K.-E.: The thrombin generation test in the diagnosis of classical hemophilia and Christmas disease. Scandinav. J. Clin. & Lab. Investigation *8*, 138, 1956 a.

— Deficiency of a factor in the plasma thromboplastin system revealed by freezing of the plasma. Dan. med. Bull. *3*, 222, 1956 b.

— Deficiency of the Hageman Factor demonstrated by the thrombin generation test. Thromb. Dith. Haemorrh. *1*, 153, 1957 a.

— Classical haemophilia (AHF deficiency) and Christmas factor (PTC) deficiency as simultaneous defects. Acta med. Scandinav. *159*, 7, 1957 b.

— The Hageman trait in a family with haemorrhagic diathesis. Acta Genetica et Statistica Medica. *7*, 541, 1958 a.

— Variations of the coagulation defects in haemophilia. Proc. 7th International Congress of Hematology, Rome 1958 b.

— Haemophilic diseases in Denmark. A classification of the clotting defects in 78 haemophilic families. (Thesis 1960) Blackwell Scientific Publications, Oxford, 1960.

Voss, D. & Waaler, B. A.: Congenital hypoproconvertinemia. A report on 12 cases with total deficiency and 19 cases with partial deficiency. Thromb. Diath. haemorrh. *3*, 375, 1959.

Scandinav. J. Clin. & Lab. Investigation 390—395, 13, 1961.

FLUOROMETRIC DETERMINATION OF TRYPTAMINE IN URINE WITH THE ZEISS ULTRAVIOLET SPECTROPHOTOMETER-FLUOROMETER

By L.-G. ALLGÉN*, K. E. FUNKE** and B. NAUCKHOFF*

From the Department of Clinical Chemistry, Beckomberga Mental Hospital, Bromma and Zeiss Svenska AB, Stockholm**, Sweden*

(Received for publication December 21, 1960)

Tryptamine was shown by Rodnight (1956) to be a normal constituent of urine. In recent studies on amine oxidase inhibition, the tryptamine content was found by Hess, Redfield & Udenfriend (1959) to increase in the tissues (brain and liver) of experimental animals and in addition a markedly elevated output of tryptamine in urine was found in humans (Oates & Zaltzman, 1959, Sjoerdsma, Oates, Zaltzman & Udenfriend, 1959) during treatment with amine oxidase inhibitors. In fact the urinary tryptamine excretion constituted a very sensitive index of amine oxidase inhibition.

The last mentioned authors described a method for the assay of urinary tryptamine using spectrophotofluorometry, with a specialized instrument (Aminco-Bowman). The present report is an adaption of the method for assay with a fluorometer. At the same time, a test of the performance of the fluorometer in the ultraviolet region is obtained.

METHODS

Extraction of tryptamine.

The procedure given by Sjoerdsma, Oates, Zaltzman & Udenfriend (1959) was employed. The volume of 0.1 N HCl solution used for the f extraction was increased to 2.0 ml, however, 1.0 ml of the extract was diluted with 2.0 ml borate buffer for fluorometric assay. Tryptam HCl (Hoffmann-La Roche) was used as stand (mol.weight 197).

Instruments.

The Zeiss spectrophotometer mod. PMQ II v monochromator M4 Q II was used with the ada for fluorometry, ZFM 4, including mercury l (Heraeus St 41), condensor lens system and ph multiplier tube (RCA IP 28). Quartz optics w used throughout.

Absorbance curves were determined with Beckman mod. DK-2 recording spectrophotom

EXPERIMENTAL RESULTS AND DISCUSSION

Optical properties of tryptamine.

Part of the U.V. absorbance curve of t tamine in aqueous solution is given in Fi It is independent of pH in the pH r 1—10. A main absorbance peak is obta around 220 nm and a lower system of p around 280 nm. Sjoerdsma, Oates, Z man & Udenfriend reported an activa

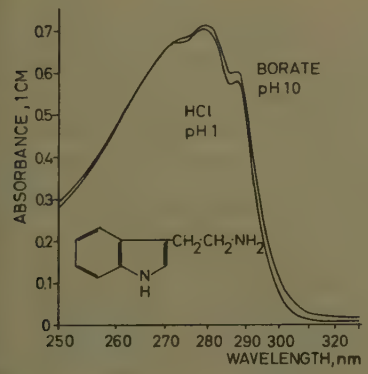
ximum at 285 nm and a fluorescence ximum at 360 nm in aqueous solution at I 10. The activation maximum corresponds the absorbance peaks around 280 nm.

lection of mercury line.

The Hg lamp does not give a continuous ectral distribution of energy, but a series spectral lines, some of which are given in ble I. Around 280 nm only low energy es are present, among which those at 5.2, 280.4 and 289.4 nm are the more in- se and were selected for activation experi- nts with tryptamine.

termination of fluorescence maximum.

To obtain the wavelength distribution of emitted fluorescence light the instrument up in Fig. 2 was used. This included two nochromators for the selection of activ- ng Hg line ($M_{prim.}$) and wavelength ana-



. 1. Ultraviolet absorbance curves for tryptamine he 280 nm region. 25 mg/l in borate buffer pH and 0.1 *N* HCl respectively. Measurements with Beckman modell DK-2 recording spectrophoto- er with the respective solvents as reference solutions.

Table I. *Some spectral lines from the Heraeus St 41 medium pressure mercury lamp.*

Wavelength nm	Relative Intensity	Wavelength nm	Relative Intensity
579.1	14	334.1	25
577.0	13	313.2	62
546.1	56	302.2	42
491.6	1.1	296.7	32
435.8	100	292.5	3.8
407.8	13	289.4	14.9
404.7	74	280.4	6.9
390.6	1.3	275.3	6.4
365.5	60	269.9	1.7
365.4	109	265.2	13.3
365.0	120	237.8	1.2

The values in the table were obtained with the Zeiss PMQ II spectrophotometer with a fixed slit width of 0.0033 mm. Thus the band width increased with the wavelength. No corrections were made for the variation with the wavelength of the dispersion of quartz and the sensitivity of the photomultiplier tube (IP 28). Thus the intensities at wavelengths below about 300 nm and at 577 and 579 nm are too low.

lysis of the emitted light ($M_{sec.}$), thus constituting a complete spectrofluorometer. Urines, standard solutions of tryptamine and reagent blanks were analysed after passing through the extraction procedure (final pH 10). Some results are given in Fig. 3. In agreement with the results obtained by Sjoerdsma, Oates, Zaltzman & Udenfriend, a fluorescence maximum was obtained at 360 nm and maximum activation was obtained at the 280.4 nm line if the difference in relative intensity for the 3 lines studied is taken into consideration. Practically the 265.2 nm line is more efficient, however, as is obvious from Fig. 3. The 289.4 line was not more efficient than the 280.4 nm line in spite of the higher relative intensity. The

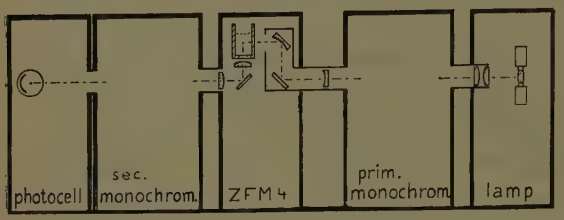


Fig. 2. Instrument setup for spectrophotofluorometry. Mercury lamp: St 41, primary and secondary monochromators: Zeiss M4 Q II, phototube: IP 28.

fluorescence curves for the urinary extracts are closely similar to the curve for tryptamine standard, both on analysis of normal human urine (curve I) and during treatment with an amine oxidase inhibitor, β-phenylisopropylhydrazine, Catron ® (curve II).

Simplified instrument setup.

As optical filters are available in the long wave U.V. region from about 310 nm, it was possible to simplify the instrument setup so that only one monochromator was used, viz: for the selection of the activating wavelength ($M_{prim.}$) whereas the secondary monochromator was replaced by a filter (Fig. 4). The most suitable filter was found to be Schott UG 4 (2 mm) with a transmission maximum at 360 nm. Several combinations of activating wavelengths and filters were tried and the 280.4 Hg line with filter UG 4 was found to give the best relation between the fluorescence intensity for sample and blank, and the highest specificity on analysis of biological samples. As mentioned above, somewhat higher sensitivity was obtained when using the 265.2 nm line but then the specificity was lower. To obtain a high degree of specificity in the fluorometric determination it is desirable to work as close as possible to the activation and fluorescen maxima. This goal is reached fairly well wi the selection of Hg line and filter made. further check of the activation maximu was made by measurements using the "co tinuous" spectral emission from a hydrog lamp (H 30) instead of the mercury lam with instrument setup as in Fig. 4 and filt UG 4. The activation maximum was tained at 280 nm (Fig. 5).

Sensitivity.

The sensitivity of the instrument w sufficient for the determination of the l tryptamine content in normal urine (usua below 0.2 μg/ml ≃ 1 μM/l as tryptami HCl) and still the sensitivity may be creased 10 times by using maximum el tronic amplification. In general a slit wi

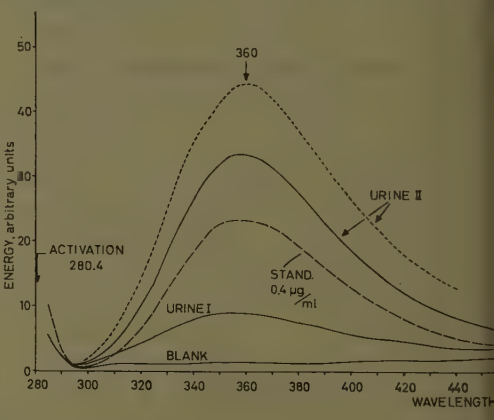
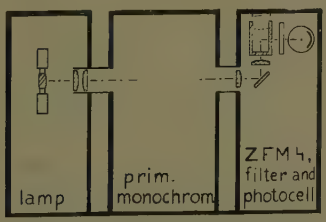


Fig. 3. Fluorescence curves obtained for tryptam solutions in borate buffer pH 10 using activat at the 280.4 nm Hg line and instrument setup in Fig. 2. All solutions have passed the extract procedure given in the text. The curves for ur extracts were obtained from the same patient bef (I) and during (II) treatment with the amine idase inhibitor β-phenylisopropylhydrazine. upper curve (II, broken) was obtained on acti tion at 265.2 nm.

0.10—0.20 mm was used. This may be creased considerably without too much loss specificity, and thus sensitivity may be creased further.

The activating efficiency of the 40 W Hg mp at 280.4 nm was found to be about 360 nes higher than that of the hydrogen lamp the same wavelength. This means that the g lamp — at this weak U.V. line — is as ficient as the 150 W xenon lamps generally ed for spectrophotofluorometry. The ad-ntage of xenon lamps is the "continuous" pe of high energy spectral emission, but a nitation is the low degree of stability of e emission intensity. The Hg lamp emis-on is much more stable and the somewhat nexpected finding that the emission inten-y at the "weak" 280.4 nm line compares vorably with the xenon lamps is promising r the determination of low concentrations U.V. fluorescent compounds (see, *e. g.*, denfriend, Duggan, Vasta & Brodie, 1957, d Duggan, Bowman, Brodie & Uden-end, 1957).

With the simplified instrument setup the eful concentration region for tryptamine alysis was found to be about 0.003-3 μl/ml the measuring solution. This corresponds a concentration range of about 0.002— ug/ml in the sample analysed with the ex-



g. 4. Simplified instrument setup for photofluoro-try. The secondary monochromator in Fig. 2 has been replaced by a filter.

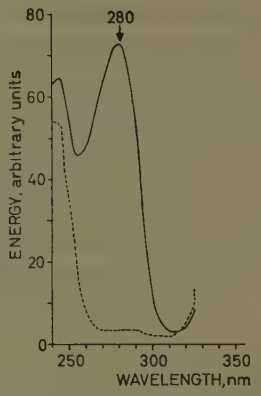


Fig. 5. Activation maximum for tryptamine (0.3 mg/l) in borate buffer pH 10, determined with instrument setup as in Fig. 4 and filter UG 4 and using a hydrogen lamp (H 30). The broken curve was obtained for a borate blank solution.

tration procedure used. For the analysis of urine before and during treatment with monoamine oxidase inhibitors tryptamine standards containing 0.4 and 0.1 μg/ml and a blank (water) were included in each measuring series and were passed through the extraction procedure. Accuracy may be further increased by the use of internal standard added to each sample.

Within the concentration region given, a linear relationship between concentration and fluorescence intensity was obtained. At higher concentrations self-quenching was noticed. The lower concentration limit will depend on the purity of the reagents used, as the blank reading will be high when maximum instrumental sensitivity is employed.

Interference.

On irradiation of tryptamine solutions at pH 10 and pH 1 with short wave U.V. light

Table II. *Influence of potential interfering materials on fluorometric analysis using the procedure for tryptamine without and with extraction.*

Material	Relative scale reading, corr., per μM/l	
	directly	after extaction for tryptamine
Indoles		
Tryptamine	100	100
Serotonin[1]	60.5	0
N,N-dimethyl-tryptamine[2]	35.9	41.8
Indole-3-acetic acid[3]	132.5	0
5-Hydroxyindole-3-acetic acid[4]	94.0	0
D,L-Tryptohan[5]	45.4	0
D,L-5-Hydroxy-tryptophan[6]	53.4	0
p-Tyramine[7]	1.2	1.2
Quinidine[8]	38.3	57.3
Isopropylisonicotinoyl-hydrazine[9]	0	–
β-Phenylisopropyl-hydrazine[10]	0	–

[1] creatinine sulfate (Sigma Chem. Co.) [2] hydrogen oxalate (California Corp. of Biochem. Research), [3]) [5]) [6]) (Sigma), [4] cyclohexylamine salt (Calif. Corp.), [7] HCl (Hoffmann-La Roche), [8] sulfate (Ph. Suecica), [9] phosphate (Marsilid®, Roche), [10] HCl (Catron®, Lakeside).

(around 250 nm), rapid breakdown of tryptamine was observed (studied by U.V absorbance, fluorometry and paper chromatography). On irradiation at 280 nm or at longer wavelengths for one hour no such effects were observed, however, when intensities comparable to those used in fluorometric assay were employed.

Several indole compounds show fluorescence properties similar to tryptamine (Duggan, Bowman, Brodie & Udenfriend). This is obvious from Table II. With the extraction procedure used, the interference from th acidic indole compounds and also from ser tonin is eliminated. N,N-dimethyltryptamin will interfere, but methylated tryptamines not seem to be present in human urine. As further check of the analytical procedur extracts in 0.1 N hydrochloric acid obtain with the extraction method used for fluor metry, were evaporated to dryness and an lysed by two-dimensional paper chromat graphy (according to Jepson, 1956) in is propanol-ammonia (I), butanol-acetic ac (II). On development with Ehrlich's re gent, tryptamine was the only indole co pound observed. In addition small amoun of U.V.-absorbing material were also se (tyramines ?). Tyramine has different fluo escence characteristics and will not interfe in the fluorometric assay of tryptamin (Table II). In one case treated with qui idine sulfate the apparent content of try tamine in urine was very high. This w shown to be due to the presence of qui idine in the urine (activation and fluo escence curves, paper chromatography). T amine oxidase inhibitors isopropylisonic tinoylhydrazine (Marsilid ®) and β-pheny isopropylhydrazine (Catron ®) do not inte fere in the determination of tryptamine.

With the simplified instrument setup qualitative check on the identity with trypt mine of the material determined is obtain Therefore it may only be used under co trolled experimental conditions, *i. e.*, wh the patient does not receive any drugs when drugs given (or metabolites fr drugs) have been shown not to interfere the determination. This may be checked determining activation and fluorescence c ves and/or by paper chromatography.

rmal values.

Rodnight as well as Sjoerdsma, Oates, ltzman & Udenfriend found tryptamine cretion to vary within the range 25—130 /24 hrs in normals and patients with rious somatic disorders. We find the same nge of values for normals but much wider nits for patients with various psychiatric sorders (24—256 µg/24 hrs). The concen- ıtion in urine did not exceed 0.2 µg/ml in ese two groups. On treatment with various onoamine oxidase inhibitors the urinary cretion of tryptamine was increased up to maximum of 2100 µg/24 hrs.

This study has been supported by Anton and rothea Bexelius Memorial Foundation.

REFERENCES

ggan, D. E., Bowman, R. L., Brodie, B. B. & Udenfriend, S.: A spectrophotofluorometric study of compounds of biological interest. Arch. Biochem. Biophys. *68,* 1, 1957.

Hess, S., Redfield, B. G. & Udenfriend, S.: Tryptamine in animal tissues following administration of iproniazid. Federation Proc. *18,* 402, 1959.

Jepson, J. B.: Paper chromatography of urinary indoles. Lancet *II,* 1009, 1955.

Oates, J. A. & Zaltzman, P.: Discussion. Symposium on amine oxidase inhibitors. Ann N. Y. Acad. Sc. *80,* 977, 1959.

Rodnight, R.: Separation and characterization of urinary indoles resembling 5-hydroxytryptamine and tryptamine. Biochem. J. *64,* 621, 1956.

Sjoerdsma, A., Oates, J. A., Zaltzman, P. & Udenfriend, S.: Identification and assay of urinary tryptamine. Application as an index of monoamine oxidase inhibition in man. J. Pharmacol. & Exper. Therap. *126,* 217, 1959.

Udenfriend, S., Duggan, D. E., Vasta, B. M. & Brodie, B. B.: A spectrophotofluorometric study of organic compounds of pharmacological interest. J. Pharmacol. & Exper. Therap. *120,* 26, 1957.

Scandinav. J. Clin. & Lab. Investigation 396—401, 13, 1961.

PAPER-ELECTROPHORETIC STUDIES ON HUMAN SEME WITH SPECIAL INTEREST IN THE DISTRIBUTION OF ADMINISTERED ZN^{65} [1]

By K. EKBOM and B. WETTERDAL

From the Urological Section of the Department of Surgery, St. Erik's Hospital, Stockholm, Sweden.

(Received for publication December 22, 1960)

The proteins of human semen have previously attracted little attention. The total protein content in the seminal plasma has been calculated to be about 3.5 to 5.5 per cent, (Huggins, Scott & Heinen, 1942). In some early papers 4 to 5 protein components could be separated by means of free electrophoresis (Gray & Huggins, 1942; Ross, Moore & Miller, 1942). Larson & Salisbury (1954) demonstrated 11 fractions in bovine seminal plasma, most of them similar to the alpha-globulins of the blood plasma.

In recent years, however, a number of publications have appeared about electrophoretic separations of semen on different mediums. Paper (Keller & Tschumi, 1953; Obé & Hermann, 1954; Schneider, Nowakowski & Voigt, 1954; Camba & Carta, 1954; Paolella, 1957; Fiala & Raboch, 1958; Keutel & Gabsch, 1958; Louros, Kaskarelis & Gnafakis, 1960), agar (Hermann, Licht, Keutel & Krug, 1958; Hermann, 1958) and starch-gel (Estborn Swedin, 1959; Estborn, 1959) have be used. The results indicate a heterogeneo composition of the seminal proteins wi generally 5 to 7 fractions. The distinction up to 13 fractions has been reported Camba & Carta (1954). The identificati of the proteins is not yet completely esta lished. Albumin is considered to be prese in small quantities or to be absent in t seminal plasma. The electropherograms see to be subjected to considerable changes carried out after prolonged storing of t ejaculates. Some authors claim to have o served differences between normal and path logic samples. The findings from the vario investigations are difficult to compare because of the many different separati techniques used.

The occurrence of zinc in the male rep ductive organs was first demonstrated a discussed by Bertrand & Vladesco (192 Since then numerous papers have appear on zinc in the male reproductive organs. T spermatozoa and the prostatic secretion ha been found to have an especially high c

[1] The investigation was supported by a grant from the foundation "Stiftelsen Thérèse och Johan Anderssons Minne".

tration of zinc in human semen (Mawson Fischer, 1953). Observations by Wetter- (1958) indicate that the spermatozoa e up zinc in the seminiferous tubuli of testis and with a relatively large content zinc are transported through the sperm nsport system to the ejaculate. Very little ms at present to be known of the import- ce of this zinc. No reports of the localiza- n of zinc in the proteins of human semen m to have appeared in earlier literature.

EXPERIMENTAL TECHNIQUES

terial.

Freshly obtained ejaculates from 42 different ividuals were subjected to paper electrophoretic dies.[2]. Experiments with radioactive zinc were ried out on 25 ejaculates. In 5 cases studies were formed on seminal plasma freed of spermatozoa. cording to the degree of fertility in this material classification of Leeb (1955) was preferred, ere group I consists of fertile, group II of sub- tile, group III of definitely infertile and group of azoospermic ejaculates.

To obtain seminal plasma free of spermatozoa a thod recommended by Lagergren was used. The nen was carefully poured upon 60 per cent glyc- n and centrifugated for 30 minutes at 3000 rpm. e supernatant contained seminal plasma and was croscopically checked to be free of spermatozoa.

per electrophoresis.

Electrophoresis on paper was preferred to other diums as autoradiographs from paper are the iest to obtain.

The ejaculates were stored at + 4° C until jected to paper electrophoretic analysis which s carried out 5 to 6 hours after the ejaculation.

[2] We are indebted to Dr. C. G. Lagergren, head the Sterility Laboratory, Department of Obste- cs and Gynecology, Sabbatsbergs sjukhus, Stock- m, for obtaining the ejaculates and for the micro- pical analyses.

Table I. *The composition of the material.*

Material	Experimental technique	Degree of fertility				Total
		I	II	III	IV	
ejaculate	paper electrophoresis	23	12	5	2	42
ejaculate	paper electrophoresis with Zn^{65}	14	5	5	1	25
seminal plasma	paper electrophoresis with and without Zn^{65}	2	2	1	0	5

Horizontal strip cabinets, as described by Dettker & Andurén (1954), and No. 1 Whatman filter papers were used. The running time was 8 hours and the voltage 220 V. (The filter papers held a potential gradient of 6.3 V/cm.) The buffer solution used had a pH of 8.6 and its composition was as follows: Veronal sodium 91 g, 1-N hydrochloric acid 60 ml, barium acetate 4.8 g, ethylene glycol 450 ml and aq. dest. to a 5000 ml dilution. This buffer solution, which is slightly modified after Laurell, Laurell & Skoog (1956),[3] gives a satisfactory separation of the β-1 and β-2 fractions in blood serum. The strips were stained in Amido-schwarz.

Radioactive zinc, Zn^{65}, and its determinations.

The original radioactive zinc, Zn^{65}, was obtained from the Biochemical Centre, Amersham, England, as a Zn^{65} Cl_2 solution. The specific activity of this solution was 1.055 mC/mg Zn. The original isotope solution was diluted with 0.85 per cent sodium chloride and 0.01 ml of this solution was added to 0.5 ml of ejaculate at room temperature about 3 to

[3] The modification, introduced by the Central laboratory of this hospital, consists of an exchange of calcium acetate, used by Laurell et al., for barium acetate.

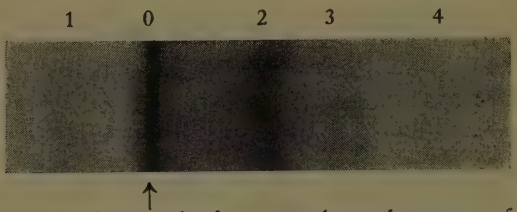


Fig. 1. Photograph of a paper electropherogram of human semen illustrating the different fractions. Arrow indicates origin.

4 hours after the ejaculation and thoroughly mixed. 0.02 ml of this ejaculate was stored for 2 hours at room temperature containing 0.315 μC Zn^{65} and then subjected to paper electrophoresis with the same technique as already mentioned. The radioactivity of the strips was studied by scintillation measurements and by autoradiography and compared with the corresponding electropherogram.

The scintillation detector used was a Tracerlab P-20 AQG unit operated with a Tracerlab Autoscaler. The strips were cut into 4 mm pieces and counted separately under identical conditions.

For the autoradiographic studies a high speed film such as Gaevert no-screen X-ray film was used. The apposition technique was preferred and the exposure technique was carried out in accordance with the descriptions of Odeblad (1952). After exposure for 10 days the films were developed in Gaevert 209 A at 18—20° C and fixed in Gaevert Acido Fix. The films were finally washed in running water and dried in air at room temperature.

RESULTS

The electrophoretic pattern.

It was possible to separate 5 well-defined fractions which for practical reasons were numbered from 0 to 4. The fraction which remained at the starting line was called the 0 fraction. An electropherogram which is considered to present normal patterns is shown in Fig. 1. One band, the 1st, is seen to migrate towards the cathode, the 0 fraction remains at the application point and the 2nd, the 3rd and the 4th fractions move towar the anode.

Inconstantly, one to two fractions appea between the 0 fraction and the 2nd fracti and in some electropherograms one com nent was seen between the 0 and the fraction migrating towards the cathode. to 8 separate components of the electrophe gram could thus be demonstrated. Th samples completely lacked the catho fractions. No protein component correspo ing to albumin in blood plasma could demonstrated.

As a rule it was difficult to recognize a correlation between the appearance of electropherogram and the morphologi classification of the corresponding ejacula The three electropherograms lacking catho fractions, however, were all from pathol ical ejaculates, one being an azoospermia, definitely infertile and one subfertile. So other quantitative variations in the differ protein components were observed in path logical ejaculates but these variati occurred inconstantly and were not cons ered to be pathognomonic.

The electropherograms could not be s to be significantly influenced by storing ejaculates at room temperature or at + 4°

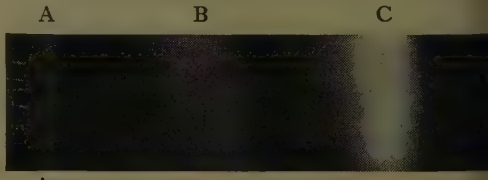


Fig. 2. Autoradiograph of a paper electropherog of human semen incubated with Zn^{65} illustrating different zones. The picture is a negative p where the white areas represent concentration radioactivity.

localization of Zn^{65}.

ig. 2 shows a picture of an autoradio-
ph which is considered to demonstrate
nal patterns. The autoradiograph is ob-
ed from an electropherogram of a normal
ulate incubated with radioactive zinc.
he main part of the activity is seen to be
lized in three zones, which were named
A, the B and the C zone. The A zone
ears to be faint and corresponds to the 0
tion in the electropherogram. The B zone
onstrates a stronger activity and appears
he region of the 2nd fraction. The C zone
ws the strongest activity and appears
ewhat closer to the anode than the 4th
tion in the electropherogram. The char-
r of the C zone was not apparently in-
nced by variations in the protein patterns
the paper electropherograms. This fact
the localization of the C zone seem to
icate that it is due to the migration of
Zn^{65} ions.

Little difference appeared in the autoradio-
phs of normal and pathological samples.
autoradiographs did not seem to be sig-
cantly influenced by the number or the
racter of the spermatozoa present in the
culate. Thus normal ejaculates and semi-
plasma, freed of spermatozoa by centri-
ation, presented autoradiographs without
significant differences.

Experiments were also performed to study
correlation of zinc to the lipid materials
the ejaculate. Chloroform was added to
normal ejaculate incubated with Zn^{65}.
er extraction, the chloroform phase was
jected to scintillation measurements and
found to show almost no activity. This
icates that zinc added to the ejaculate is
attached to the lipid materials.

DISCUSSION

The heterogeneous composition of the proteins in human semen is further illustrated in this investigation. The results agree fairly well with those obtained by Obé & Hermann (1954), Keutel & Gabsch (1958) and others. Obé & Hermann (1954) also obtained electropherograms with no cathodic fractions in 3 patients with bilateral closure of the *vasa deferentia*. The absence of cathodic fractions might be due to disturbances in the testis epididymis function. In the present study it was possible to demonstrate an absence of cathodic fractions in only 3 cases of 19 ejaculates having a diminished number of or morphologically pathological spermatozoa. This further illustrates the difficulties in interpreting the electropherograms obtained.

The 0 fraction may possibly be due to proteins remaining at the point of application during the electrophoresis. No significant differences in this fraction could be demonstrated in azoospermic and normospermic ejaculates.

The three anodic fractions were not seen to differ significantly in normal and in pathological ejaculates, or the variations occurred inconstantly and were not regarded to be pathognomonic.

The 2nd fraction in the ejaculates was seen to migrate with the same speed as the β-2-globulin fraction of blood serum in electrophoresis carried out under identical conditions. Keutel & Gabsch (1958) also believed their corresponding protein fraction, the A_1 fraction, to be identical with the β-globulins.

Comparisons between electrophoretic studies on semen in different investigations are, however, difficult because of the various techniques and methods used.

From the autoradiographs obtained, added zinc appears to occur in human semen in two states, partly as free Zn ions corresponding to the C zone and partly as protein-bound zinc corresponding to the B zone. The A zone is probably due to zinc attached to proteins at the point of application. Added zinc does not seem to be bound to the lipid materials.

The present results do not admit a definite conclusion concerning the distribution of the naturally occurring zinc in the semen because we have not had the possibility to prove that the added zinc is uniformly distributed in the zinc originally present in the different fractions of the samples. This distribution might be studied in at least two different ways.

One would be to examine if changes in the zinc distribution occur after different times of incubation. Because of the rapid change of the proteins of the seminal plasma, which has been reported by several authors, the paper electrophoresis had to be performed as soon as possible after the ejaculation. This limits the time for incubation. Smaller variations of the time of incubation, however, did not seem to influence the autoradiographic patterns.

Another way to study the zinc distribution would be to examine the ratio radioactive zinc/inactive zinc in the different fractions of the semen. Such an investigation involves several difficulties. It is, however, planned to be carried out by the use of preparative electrophoresis.

Mawson & Fischer (1953) observed that in the human ejaculate much zinc was derived from the prostatic secretion. Investigations on rats by Wetterdal (1958) indicated that the spermatozoa contain a high amoun zinc as does the seminal plasma. The n part of the plasma zinc probably origin from the prostatic secretion. It seems possible that the main part of the prot bound zinc in the present investigatio derived from the prostatic secretion. I hoped that hormonal studies in this field give further information.

SUMMARY

Paper electrophoretic studies of hu semen were undertaken. It was possibl separate 5 well-defined protein fraction pH 8.6, one migrating towards the cath one remaining at the starting line and t anodic bands. There seemed to be no sig icant relationship between the appearanc the different protein components of electropherograms and the clinical pictur fertility. The absence of the cathodic f tions, however, might indicate a disturb of the reproductive function resulting in grade fertility.

In an attempt to examine the distribu of zinc in the different fractions of hu seminal fluid, samples of this fluid fron men were incubated with radioactive and subjected to electrophoresis. By m of autoradiography and scintillation m urements, part of the zinc added to the ulate was found to migrate together wi protein component with the same spee β-2-globulin of blood serum. This "pro bound" zinc could not be shown to be si icantly influenced by the number or the phology of the spermatozoa. Part of added zinc in the ejaculate seems to exi a free state.

REFERENCES

'and, G. & Vladesco, R.: Sur la variation de a teneur en zinc de l'organisme du lapin durant a croissance. Compt. rend. Acad. d.sc. *173,* 54, 921.

ba, R. & Carta, R.: Studi elettroforetici sul lasma seminale umano. Arch. De Vecchi Anat. pat. *22,* 565, 1954.

ter, A. & Andurén, A.: A new time-saving pparatus for paper electrophoresis. Scandinav. . Clin. & Lab. Investigation *6,* 74, 1954.

rn, B. & Swedin, B.: Localisation of acid hosphatase by starch-gel electrophoresis of ractionally collected human semen. Scandinav. . Clin. & Lab. Investigation *11,* 235, 1959.

Visualization of Acid and Alkaline Phosphat- se after Starch-Gel Electrophoresis of Seminal Plasma, Serum and Bile. Nature *184,* Suppl. 21, 1636, 1959.

, J. & Raboch, J.: Papirová elektroforesa ílkovin v lidském ejakulátu. Cas. lék ces. 97, 240, 1958.

, S. & Huggins, C.: Electrophoretic analysis f human semen. Proc. Soc. Exper. Biol. & Med. *50,* 351, 1942.

ann, G., Licht, W., Keutel, H. J. & Krug, E.: Elektrophoretische Untersuchungen am enschlichen Spermaplasma. Ztschr. f. d. ges. xper. Med. *130,* 436, 1958.

mmunoelektrophoretische Untersuchungen am enschlichen Spermaplasma. Clin. chim. acta , 116, 1959.

gins, C., Scott, W. W. & Heinen, J. H.: Chemical composition of human semen and of ecretions of prostate and seminal vesicles. Am. . Physiol. *136,* 467, 1942.

r, M. & Tschumi, R.: Elektrophoretische Studien am menschlichen Spermaplasma. Gy- aecologia *135,* 92, 1953.

Keutel, H. J. & Gabsch, H. Ch.: Studien am menschlichen Sperma. Urol. int. *6,* 206, 1958.

Lagergren, C. G.: Personal Communication 1960.

Larson, B. L. & Salisbury, G. W.: The proteins of bovine seminal plasma. I. Preliminary and electrophoretic studies. J. Biol. Chem. *206,* 741, 1954.

Laurell, C.-B., Laurell, S. & Skoog, N.: Buffer Composition in Paper Electrophoresis. Clin. Chem. *2,* 99, 1956.

Leeb, H.: Zur Bewertung der männlichen Fertilität aus der Spermauntersuchung. Geburtsh. u. Frauenheilk. *15,* 443, 1955.

Louros, N. C., Kaskarelis, D. & Gnafakis, N.: Études sur les proteins du plasma séminal humain. Acta urol. belg. *84,* 28, 1960.

Mawson, C. A. & Fischer, M. I.: Zinc and carbonic anhydrase in Human Semen. Biochem. J. *55,* 696, 1953.

Obé, G. & Hermann, G.: Papierelektrophoretische Untersuchungen am Spermaplasma, Ejakulat und Prostataexprimat. Ztschr. f. Urol. *47,* 393, 1954.

Odeblad, E.: Contributions to the theory and technique of quantitative autoradiography with P^{32} with special reference to the granulosa tissue of the Graafian follicles in the rabbit. Acta radiol. Suppl. 93, 1952.

Paolella, A.: Elettroforesi su carta di liquido seminale umano normale e patologico. Minerva med. *77,* 48, 1957.

Ross, V., Moore, D. H. & Miller, E. G. jr.: Proteins of human seminal plasma. J. Biol. Chem. *144,* 667, 1942.

Schneider, W., Nowakowski, H. & Voigt, K. D.: Die Papierelektrophorese von menschlichem Spermaplasma. Klin. Wchnschr. *32,* 863, 1954.

Wetterdal, B.: Experimental studies on radioactive zinc in the male reproductive organs of the rat. Acta radiol. Suppl. 156, 1958.

Scandinav. J. Clin. & Lab. Investigation 402—409, 13, 1961.

AN IMPROVED METHOD FOR THE ESTIMATION OF SMALL QUANTITIES OF ALKALI-RESISTANT HEMOGLOBIN IN BLOOD

By K. KRISTOFFERSEN

From the Central Laboratory, Amtssygehuset, Århus, Denmark

(Received for publication December 27, 1960)

The distinction between fetal hemoglobin (Hb-F) and adult hemoglobin (Hb-A) is well known through the great difference in their resistance towards denaturation by strong base (Körber 1866). Hb-F is much more slowly converted into alkaline hematin and denatured globin than is Hb-A, and this forms the basis of several methods for the estimation of Hb-F. The one-minute alkali-denaturation test developed by Singer, Chernoff & Singer (1951 a)and the direct spectophotometric method of Brinkman & Jonxis (1935) have both found wide application. A number of modifications of these two principles have been proposed (Beaven, Hoch & Holiday 1951; Derrien & Laurent 1954; Künzer 1955; Jonxis & Visser; 1956; Fisher 1957).

Generally, Hb-F is identified with alkali-resistant hemoglobin (A-R-Hb). However, this assumption cannot be universally accepted, especially not when measuring small amounts of this pigment. This problem will be discussed later.

The difference in alkali-resistance is only one aspect of the many differences between Hb-F and Hb-A, which are confined to the globin moiety, both pigments containing identical heme grou

Estimations of Hb-F and Hb-A, based on ferences in alkali-resistance, antigenic prope electrophoretic and chromatographic behavior spectrophotography in the region of the tryptop band, indicate that the major pigment in red of new-born children is Hb-F in amounts ran from 50 per cent to 90 per cent of total hemog (Beaven & Gratzer 1959). The Hb-F of in decreases during the first months to assume proportions of Hb-F found in older normal chi and in adults, *i.e.* less than 2 per cent. The es tion of such low percentages of Hb-F by a denaturation methods is, however, inaccurate results obtained by different procedures show siderable dispersion.

Determination of low percentages of Hb-F interest because it opens possibilities of elucid transplacental transmission of fetal blood, i determination of increased amounts of Hb-F i maternal blood, and it has aroused attention a obstetricians and pediatricians. For this pu alkali-denaturation methods have mostly applied (Christensen, Kissmeyer-Nielsen & L Jepsen 1960). But the possible increases in in the maternal blood caused by fetal transpla bleeding will in most cases be so small that me of far higher sensitivity than the existing one needed.

The need for a method of higher sensitiv also apparent from the studies of Hb-F lev

hematological disorders (Beaven & White ; Kornerup & Faber 1956; Beaven, Ellis & te 1960).

mple physical reasoning will show that deter- tion of small proportions of Hb-F by the one- te alkali-denaturation method cannot be reli- since the absorbance at the wavelength em- d (540 nm) is too low for exact measurement. hermore, a relatively great part of the residual rbance, *i.e.* the absorbance after denaturation removal of the denatured pigment, is not rep- ted by Hb-F (Beaven *et al* 1960). Falbe-Han- (1959) has carried out spectrophotometric es, indicating that the alkali-resistant residue ined by the method of Singer *et al.* consists of al components, among them native oxyhemo- n. From these findings it follows that low ntages of Hb-F are found too high, the error asing on decreasing values.

lighly sensitive methods for determina- of Hb-F have been described, one based antigenic properties of Hb-A and F ernoff 1953 b), and another based on li-denaturation combined with trypto- ne band spectography (Beaven *et al.* 0), but the alkali-denaturation test has obvious advantage of being technically y simple. Therefore, the possibility of eloping a more sensitive and more speci- alkali-denaturation test was examined. procedure finally adopted is a modified minute alkali-denaturation method, cap- of detecting Hb-F at the 0.1 per cent l. The increased sensitivity was obtained reading the residual absorbance in the et band (412—415 nm). By altering the nique of precipitation and removal of atured pigment before the spectrophoto- ric reading of the residue, a more speci- method for the determination of Hb-F developed.

METHOD

Reagents

a. Toluene.
b. Ammonium hydroxide 0.004 per cent.
c. Denaturation reagent: N/10 sodium hydroxide.
d. Precipitating reagent: 150 g $(NH_4)_2SO_4$ are dissolved in 200 ml distilled water to make 280 ml saturated $(NH_4)_2SO_4$ solution. 10 ml N HCl and distilled water are added to make 400 ml.

Equipment

Centrifuge tubes — round bottom — 15 ml volume.

Thermostatic water-bath at 25° C ± 0.1° C.

Stopwatch.

Pipettes of 25 µl, 500 µl, 4.00 ml, and 10.00 ml.

Spectrophotometer (Zeiss spectrophotometer, model M4Q was used in these experiments).

Procedure

Hemoglobin solutions (hemolysates) are prepared from heparin blood. Plasma is discarded and the erythrocytes are washed three times with 1.2 per cent saline and lysed by adding 1.5 volume ammonium hydroxide and 0.5 volume toluene, shaking vigorously for 10 minutes. After hard centrifugation (2000 G for 20 minutes), the two upper layers are discarded.

500 µl hemolysate are added to 4.00 ml NaOH, preheated to 25° C. After one minute (stopwatch), 8.50 ml precipitating reagent are added and the tube is inverted six times and placed in a refrigerator for at least one hour before centrifugation at 2000 G for one hour.

The absorbance (A_1) in the supernatant is read at the maximum (413 nm) of the Soret band in 20 mm cuvettes. For determination of the absorbance before denaturation (A_0), the hemolysate is diluted about 4000 times (D) with a mixture of 8 volumes NaOH, 17 volumes precipitating reagent and one volume distilled water (diluting mixture), which also serves as blank.

The proportion of A-R-Hb in per cent of total Hb is calculated on the basis of the equation:

$$\frac{A_1 \times 26 \times 100}{A_0 \times D} = \text{per cent A-R-Hb}$$

D expresses how many times the hemolysate is diluted for determining A_0.

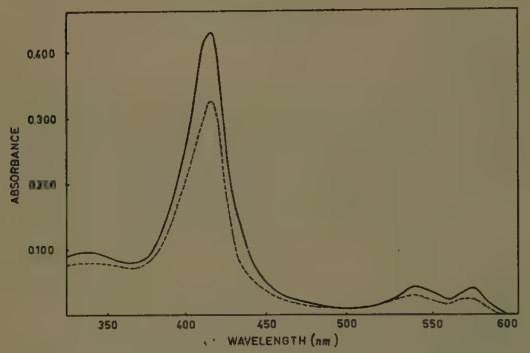


Fig. 1. Absorption curve of native hemoglobin, region 325 to 600 nm (————), and of the one-minute residual hemoglobin from blood containing 0.8 per cent A-R-Hb (------).

Accuracy

The standard deviation:

$$\text{S. D.} = \sqrt{\frac{\sum \frac{n}{1} \cdot d^2}{2n}}$$

calculated from 100 double analyses is found to be ± 0.012 per cent for the values of A-R-Hb ranging from 0.07 per cent to 1.25 per cent.

The following recovery experiments were performed:

Different small amounts of hemolysate from cord blood containing 55.5 per cent A-R-Hb were added to each of 13 hemolysates from adults. The percentages of A-R-Hb were determined before and after the addition of cord blood hemolysate. The amounts of added A-R-Hb were calculated at 0.17 per cent — 0.74 per cent of total hemoglobin, and the corresponding recovered amounts ranged between 0.16 and 0.75 per cent. The results recorded in Table II show good agreement between calculated and recovered proportions of A-R-Hb. All values recorded are the mean values of duplicate determinations.

EXPERIMENTAL

A. Choice of wavelength

The Soret band maximum was chosen because the absorbance of hemoglobin is here about 9 times that of the visible maxi (Harboe 1959). The absorption maximum oxyhemoglobin lies at 412—415 nm (J 1949; Hunter 1951; Beznak 1953; Har 1949). Fig. 1 shows an absorbance curve native hemoglobin with maximum at 413 n

B. Precipitation and removal of denatu hemoglobin

It is of essential importance that the pr pitation procedure and the removal of de tured hemoglobin are complete without c comitant precipitation or removal of na hemoglobin. For removal of the precipit filtration and centrifugation were examin Filtration was found unsuitable, since a r tively high proportion of diluted hemoglo solutions from 1 to 60 mg/100 ml was fo to be adsorbed on various filters. In Tab the results of these experiments are pres ed, together with the effect of centrifugat on diluted hemoglobin solutions. It will seen that centrifugation is the method choice for removing the precipitate with loss of native hemoglobin. This conclus agrees with that of Fisher (1957).

The influence of the ammonium sulph concentration on the degree of precipita of denatured hemoglobin and of denatu hemoglobin + a small amount of A-R added from cord blood was also stud Denatured hemoglobin was prepared f adult hemoglobin by denaturation for hour.

The pH of the final solutions was between 7.3 and 7.5, by altering the volu of HCl in the precipitating reagents in cordance with the varying ammonium phate concentrations.

The results of the experiments are sh

ig. 2, in which the residual absorbance inate) are plotted against ammonium ıate concentrations in the final solutions cissa). The precipitation of denatured

le I. *The absorbances of hemoglobin solu- before and after filtration (A: Columns nd III) with different filters, and before after hard centrifugation (B: Columns nd III). The solutions with hemoglobin entrations of 1 to 10 mg/100 ml are read 13 nm and those with hemoglobin concen- ons of 10 to 60 mg/100 ml at 540 nm. percentage losses of hemoglobin by fil- on and centrifugation are recorded (A and B: Column IV.)*

el- th	II Absorbance before Filtration	III Absorbance after Filtration	IV Loss of Hb by Filtration (per cent)
0	0.432	0.380	12
0	0.515	0.440	13
0	0.035	0.017	51
0	0.037	0.016	57
3	0.320	0.282	12
3	0.134	0.100	25
3	0.084	0.054	36
3	0.470	0.243	48
3	0.485	0.245	49
3	0.433	0.124	71

	Before Centri- fugation	after Centri- fugation	Loss of Hb by Centri- fugation
0	0.320	0.320	0
0	0.318	0.316	0.6
0	0.322	0.318	1.3
3	0.590	0.595	—0.8
3	0.598	0.605	—1.2
3	0.590	0.595	—0.8
3	0.600	0.590	1.7

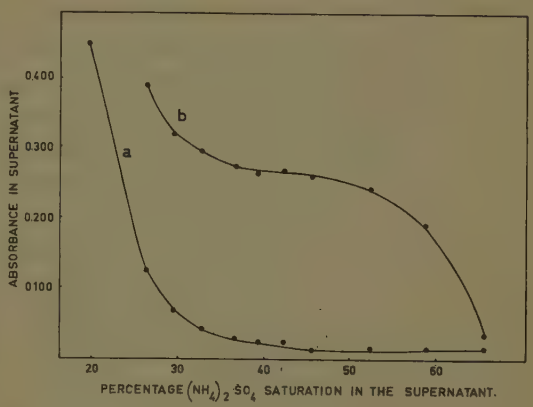


Fig. 2. *a.* Residual absorbance (ordinate) plotted against percentages of $(NH_4)_2SO_4$-saturation (abscissa) in the supernatant after precipitation and hard centrifugation. pH 7.3 to 7.5.

b. Same experiment as in *a*, except that 0.7 per cent A-R-Hb was added from cord blood.

hemoglobin (Fig. 2, curve a) increases until about 40 per cent saturation and is then constant and practically complete. Fig. 2, curve b, represents the results of precipitation of denatured hemoglobin + 0.7 per cent A-R-Hb. It will be seen that no native hemoglobin is precipitated until the saturation is close to 50 per cent. On the basis of the above it is concluded that a saturation about 45 per cent will give the most complete precipitation of denatured hemoglobin without precipitation of native hemoglobin. For practical reasons, a 45.8 per cent saturation with ammonium sulphate was chosen, as this degree of saturation will results from a mixture of 4 ml NaOH + 0.5 ml hemolysate + 8.5 ml precipitating reagent (70 per cent saturated ammonium sulphate).

From Fig. 2, curve a, it appears that the precipitation of denatured hemoglobin at

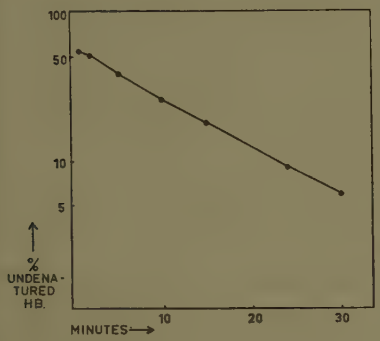
Table II. *The results of 13 recovery experiments. To each hemolysate from normal adults was added a calculated amount of A-R-Hb from cord blood (column V). The proportions of A-R-Hb were determined before (column II) and after (column III) the addition of A-R-Hb and the differences are recorded (column IV). The A-R-Hb recovered in per cent of calculated are recorded (column VI).*

I Blood sample No.	II A-R-Hb in blood before adding cord blood % of total	III A-R-Hb in blood after adding cord blood % of total	IV Difference of A-R-Hb before and after adding cord blood % of total	V Calculated per cent of A-R-Hb added to blood sample	VI A-R-Hb recovered in per cent of calculated
1	0.17	0.33	0.16	0.17	94
2	0.08	0.29	0.21	0.19	111
3	0.33	0.50	0.17	0.19	89
4	0.17	0.58	0.41	0.44	93
5	0.20	0.71	0.51	0.55	93
6	0.43	0.82	0.39	0.43	91
7	0.27	0.85	0.58	0.61	95
8	0.50	0.85	0.35	0.39	90
9	0.23	0.98	0.75	0.74	101
10	0.07	0.62	0.55	0.61	90
11	0.05	0.21	0.16	0.15	107
12	0.09	0.34	0.25	0.22	114
13	0.10	0.36	0.26	0.24	108
					Mean: 98

45.8 per cent saturation is practically complete, and the mean residual absorbance from 10 experiments at this saturation was found to be 0.013, representing 0.025 per cent ± 0.0047 per cent of the absorbances before denaturation.

C. Studies on the residue

The residue after one minute's alkali-denaturation (one minute's residue) of hemolysates from cord blood, adult blood and mixtures of these are studied by determination of alkali-denaturation rate and absorption curve. The denaturation-rate is determined by the present method with denaturation times varying from one to thirty minutes for cord blood at intervals depicted (Fig. 3), and from one to thre four minutes for 6 adult hemolysates and 5 mixtures of adult and cord blood he lysates at one minute intervals (Fig. 4). percentages of unchanged hemoglobin (c nate) are plotted logarithmically aga time (abscissa). The one minute's residu cord blood was found to be 55.5 per cen total hemoglobin, and the one minu residues in Fig. 4 ranged from 0.08 to per cent of the total. In Fig. 5 the cu from Fig. 3 and Fig. 4 are depicted wit values of one minute's residues transfe to the same point, in order to compare slopes of the curves.



g. 3. Alkali-denaturation rate plots for cord blood
ch a one-minute residual hemoglobin value of
55.5 per cent.

The one minute's residues from adult
oods show a denaturation rate with same
w slope as cord blood, indicating that the
e minute's residues from adults are the
me as the major pigment from cord blood.
robably, both can be identified with Hb-F.
oreover, the experiment shows that all
kali-labile hemoglobin (Hb-A) is dena-
red in one minute.

After denaturation as described above,
lowed by precipitation and removal of
natured hemoglobin, the absorption curve
the one minute residue was examined
om 325 to 600 nm. The results are depicted
Fig. 1 (dotted line) and compared to an
sorbance-curve for native hemoglobin (un-
oken line), indicating that the two curves
e very similar.

DISCUSSION

Since its introduction by Singer, Chernoff
Singer (1951 a), the one-minute alkali-
naturation test has been extensively used
the estimation of Hb-F. It rests on the
sumption that under the particular con-
ions, all the Hb-A is denatured, whereas
the major part of Hb-F remains unchanged and is retained in solution.

The amount of residue is assumed to be a measure of the Hb-F contents of the sample, provided that the residue is not less than 2 per cent. This was suggested by Singer and co-workers, since normal adult blood gave residues of 0.5 to 1.7 per cent, and identical residues were obtained even after prolonged denaturation. They did not assume that residues below 2 per cent consisted of Hb-F. It was proved (Jonxis & Huisman 1956 b; Beaven *et al.* 1960) that by the method of Singer *et al.* low percentages (2 to 10 per cent) of Hb-F are found too high. Fisher (1957) emphasized that filtration as applied by Singer *et al.* and other researchers, is not a good method for separating the precipitated denatured hemoglobin from the remaining native hemoglobin, since adsorption of hemoglobin onto the filter also introduced errors. In the present work, the errors caused by

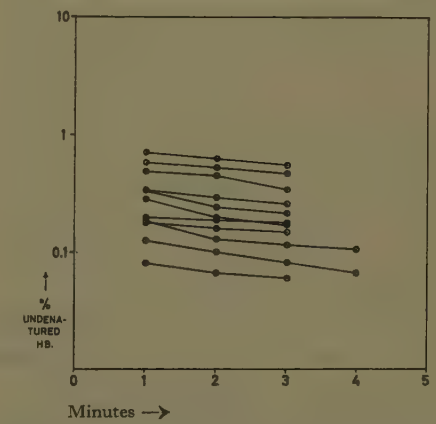


Fig. 4. Alkali-denaturation rate plots for 11 adult bloods, to 5 of which was added a small quantity of A-R-Hb from cord blood, so that the one-minute residual hemoglobin values ranged from 0.08 per cent to 0.71 per cent.

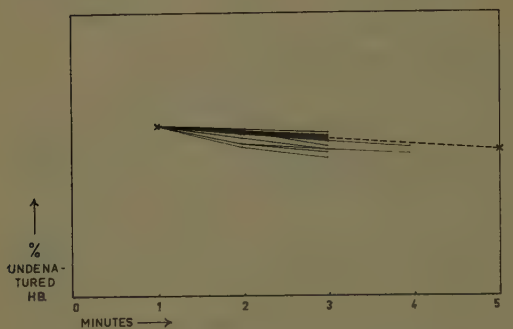


Fig. 5. Denaturation rate curves from Figs. 3 and 4, transferred to the same level, making the slopes of the curves comparable.

incomplete precipitation of denatured hemoglobin and by filtration are studied by spectrophotometric measurements in the Soret band, which has nine times higher absorbance than the visible bands (540 and 576 nm), as demonstrated in Fig. 2 and in Table I.

For precipitation of denatured hemoglobin, Singer *et al.* used 33 per cent saturation with $(NH_4)_2SO_4$ in the final solution. From the precipitation experiments shown in Fig. 2, it appears obvious that Singer's $(NH_4)_2SO_4$-concentration is too low, which explains the increase of residual hemoglobin on decreasing percentages of Hb-F.

By the present method, the proportion of residual hemoglobin, which is not native hemoglobin, is reduced to about 0.025 per cent of the total hemoglobin.

The percentage of A-R-Hb, measured by the present method, cannot be considered actually the same as the real amount of Hb-F in the blood, since a possible formation of methemoglobin-F in the hemolysate will influence the values of A-R-Hb. Betke (1954) found that by the employed method it is very difficult to obtain a hemolysate containing less than 5 per cent methemoglobin. Me hemoglobin-F is denatured just as quickly oxyhemoglobin-A, (Beaven *et al.* 1960), a consequently this oxidized pigment will n be represented in the one-minute alka denaturation residual pigment.

Moreover, a correction for the sm amount of Hb-F denatured in one minu would have to be inserted into the calcul tion by extrapolating the denaturation ra curve to zero time. With the prese technique, the alkali-denaturation rate of t major pigment from cord blood shows a r action of apparent first order kinetics for minutes, but it has not been general accepted that the alkali-denaturation rea is a reaction of first order (Betke 1954 Therefore, this correction has not been i serted in the present work.

Until the influence of methemoglobin fo mation in the hemolysates and the alka denaturation reaction have been furth elucidated, the values obtained by the pr sent method as well as by other denaturati procedures should be termed alkali-resista hemoglobin.

However, the reproducible values of A-H Hb obtained through the present recove experiments indicate that the method dev loped is more sensitive and more specific f estimation of Hb-F than other metho based on alkali-denaturation, and the meth is supposed to be applicable in investigati of transplacental feto-maternal bleeding.

SUMMARY

The development of a method suitable f the estimation of small amounts of alka resistant hemoglobin (A-R-Hb) is describe

e procedure adopted is a modification of one-minute alkali-denaturation test of ger *et al.* and is capable of detecting A-R- at the 0.1 per cent level.

The increased sensitivity was obtained by asuring at the Soret band.

By an improved technique of precipitation l removal of the denatured pigment be- e spectrophotometric reading of the resi- l unchanged hemoglobin, the method be- ne more specific for determination of -F.

ACKNOWLEDGMENT

am indebted to Professor M. Ingerslev, Århus, samples of cord blood for the experiments.

REFERENCES

ven, G. H. & Gratzer, W. B.: A critical review of human hemoglobin variants. J. Clin. Path. *12,* 1, 101, 1959.

Ellis, M. J. & White, J. C.: Studies on human foetal haemoglobin I. Brit. J. Haemat. *6,* 1, 1960.

Hoch, H. & Holiday, E. R.: The haemoglobins of the human foetus and infant. Biochem. J. *49,* 374, 1951.

& White, J. C.: Detection of foetal and sickle cell haemoglobins in human anaemies. Nature *172,* 1006, 1953.

ke, K.: Der menschliche rote Blutfarbstoff. Springer Verlag, Berlin, Göttingen, Heidelberg. 1954.

nák, M.: The light absorption of sheep hemoglobin and myoglobin compounds in the soret region. Acta chem. Scandinav. *2,* 333, 1948.

nkman, R. & Jonxis, J. H. P.: The occurrence of several kinds of haemoglobin in human blood. J. Physiol. *85,* 117, 1935.

rnoff, A. I.: Immunologic Studies of Hemoglobins. II. Quantitative precipitin test using anti-fetal hemoglobin sera. Blood *8,* 413, 1953 b.

Christensen, J., Kissmeyer-Nielsen, F. & Linnet-Jepsen, P.: Neonatal anæmi forårsaget af transplacentar blødning fra foster til moder. Ugesk. f. læger *122,* 781, 1960.

Derrien, Y. & Laurent, G.: Sur la détermination quantitative de la fraction alcalinorésistante des hémoglobines humaines. Compt. rend. Soc. de biol. *148,* 710, 1954

Falbe-Hansen, I.: Personal communcation. 1959.

Fisher, B.: Modifications in the alkali denaturation test for fetal hemoglobin. Am. J. Clin. Path. *27,* 48, 1957.

Harboe, M.: A method for determination of hemoglobin in plasma by near-ultraviolet spectrophotometry. Scandinav. J. Clin. & Lab. Investigation *11,* 66, 1959.

Hunter, F. T.: The quantitation of mixtures of hemoglobin derivates by photoelectric spectrophotometry. C. C. Thomas, Springfield. 1951.

Jonxis, J. H. P. & Huisman, T. H. J.: The detection and estimation of fetal hemoglobin by means of the alkali denaturation test. Blood *11,* 1009, 1956 b.

— & Visser, H. K. A.: Determination of low percentages of fetal hemoglobin in blood of normal children. A. M. A. J. Dis. Child. *92,* 588, 1956.

Jope, E. M.: The ultraviolet spectral absorption of hemoglobins inside and outside the red blood cell. Roughton, F. J. W. & Kendrew, J. C. (ed.). Haemoglobin, p. 205. Butterworth, London. 1949.

Kornerup, V. & Faber, M.: Alkaliresistent føtalt hæmoglobin. Nord. med. *55,* 830, 1956.

Künzer, W.: Untersuchungen über das Vorkommen fetalen Hämoglobins bei Blutkrankheiten. Ztschr. f. Kinderh. *76,* 58, 1955.

Körber, E.: Über Differenzen des Blutfarbstoffes. Zentralbl. f. d. Med. Wiss. 117, 1867. (Ref. af Dissertation, Dorpat 1866.)

Singer, K., Chernoff, A. I. & Singer, L.: Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation. Blood *4,* 413, 1951 a.

Scandinav. J. Clin. & Lab. Investigation 410—415, 13, 1961.

A FIBRINOLYTIC SYSTEM IN HUMAN PLASMA ACTIVATED BY PEPTONE OR ACID POLYSACCHARIDE

By E. S. OLESEN

From the Biological Institute of the Carlsberg Foundation, Copenhagen, Denmark

(Received for publication December 28, 1960)

Ungar & Mist (1949) and Ungar, Damgaard & Hummel (1953) observed that fibrinolytic activity appeared in isoelectrically precipitated euglobulin when guinea-pig serum, before precipitation, was incubated with peptone or various other compounds, including some of polysaccharide nature.

Personal observations (Olesen 1957, 1959 a, b, c) partially confirmed the findings of Ungar *et al.;* they indicated also that this type of activation of fibrinolysis could be the result of an interaction between anionic polyelectrolytes and an activator system in guinea-pig serum leading to release of a plasminogen activator, which in turn, acting on plasminogen, produced plasmin. Experiments on human and guinea-pig serum (Olesen 1959 b), in an attempt to produce fibrinolytic activity either by streptokinase or by a purified peptone preparation (Astrup & Olesen 1957), indicated that the 'peptone effect' in guinea-pig serum is produced by an activator system which is different from the human 'proactivator system' (in the sense of Müllertz & Lassen 1953) involved in the activation produced by streptokinase. Treatment with peptone produced no fibrinolytic activity in the human serum inve igated.

However, the negative results with ser did not rule out the possibility of a 'pepto effect' in human blood, since the necessa precursor system might have been adsorb on fibrin during clotting, or for some oth reason be absent from serum. Therefore, this study, human plasma as well as ser were investigated with respect to their abil to yield fibrinolytic activity when submitt to isoelectric precipitation in the presence peptone or acid polysaccharides.

MATERIALS AND METHODS

Human blood samples from healthy donors w collected each in two centrifuge tubes contain 1/10 volume of either a 2 per cent solution potassium oxalate or a 0.9 per cent sodium clor solution. Serum, after spontaneous coagulation, a oxalate plasma were separated by centrifugati The samples were used for experiments withi few hours, or kept at —20° for later use.

'Purified peptone' was prepared from commerc peptone (kindly supplied by the Roskilde Medi Company Ltd., Roskilde, Denmark) as described *Astrup & Olesen* (1957). *Acid polysaccharides:* heparin was kindly supplied by Messr. Hoffman-

che & Co., Basel. Cellulose trisulphuric acid dium salt), prepared according to *Astrup, Gals- & Volkert* (1944), was placed at my disposal Dr. T. Astrup.

The samples to be tested for fibrinolytic activity re prepared by isoelectric precipitation of plasma serum in the presence of either 'purified pep- e' or one of the acid polysaccharides: One lume of serum was diluted twentyfold with H_2O d added to one volume of a solution of the ac- ating compound; pH was adjusted to 5.4, and the ulting precipitate was separated by centrifuga- n. The precipitate was redissolved in one volume saline barbital buffer (0.05 M, containing 0.1 NaCl; pH 7.75). All procedures were per- med at room temperature unless otherwise ted.

Fibrinolytic activity was estimated by the fibrin te method of *Astrup & Müllertz* (1952) on 0.1 cent bovine fibrinogen clotted by bovine throm- (as kindly supplied by the Leo Pharmaceutical ., Copenhagen). Protease activity was estimated fibrin plates heated at 85° for 45 min. according *Lassen* (1952). Fibrinolytic activity is expressed the diameter product (mm^2) of the lysed zone er 18 hours incubation at 37° (average of 3 erminations). Since the susceptibility of the fibrin strates could vary from batch to batch, direct nparisons were made in simultaneous assays on rin plates prepared from the same batch of rinogen.

RESULTS

'Purified peptone'. Fig. 1 demonstrates a ical finding of fibrinolytic activity in globulin prepared by isoelectric precipita- n of human serum or plasma. Peptone oduced slight activity in serum, but con- derably higher activity in the plasma. pontaneous' activity — which here means fibrinolytic activity appearing in the sence of added compounds — was occa- nally observed in the plasma samples. This ivity was small and often recognizable

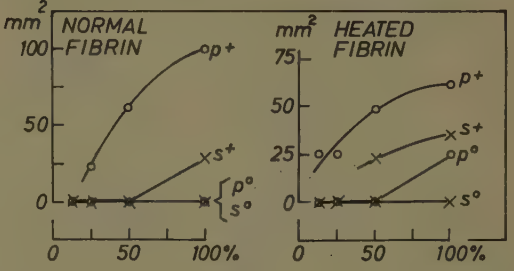


Fig. 1. Dilution curves showing the fibrinolytic activity of human serum (S) and plasma (P), after an isoelectric precipitation, either in the absence (S^0, P^0) or the presence (S^+, P^+) of 'purified peptone'. *Abscissa:* Concentration of the test solution in percentage of stock solution. *Ordinate:* Fibrinolytic activity on normal and heated fibrin as diameter product (mm^2) of lysed zones.

only on heated fibrin (as seen in Fig. 1) — probably because inhibitory substances contaminating the bovine fibrin are destroyed by heating (Lassen 1952). Lysis of heated fibrin indicated that an active protease was present in the precipitates; lysis of normal fibrin could be due also to activation of bovine plasminogen (present in the normal fibrin substrate) caused by an activator in the solutions.

β-heparin and cellulose trisulphuric acid. In experiments on guinea-pig serum, these acid polysaccharides had proven most effective in producing fibrinolytic activity (Olesen 1959 b). Results obtained on human blood with these two compounds are presented in Fig. 2. Both were able to induce some fibrinolytic activity in plasma, whereas they produced very little, or no activity in the corresponding samples of serum. The highest activities appeared at about 1/16 mg of the compounds per ml of plasma.

These results are representative for the

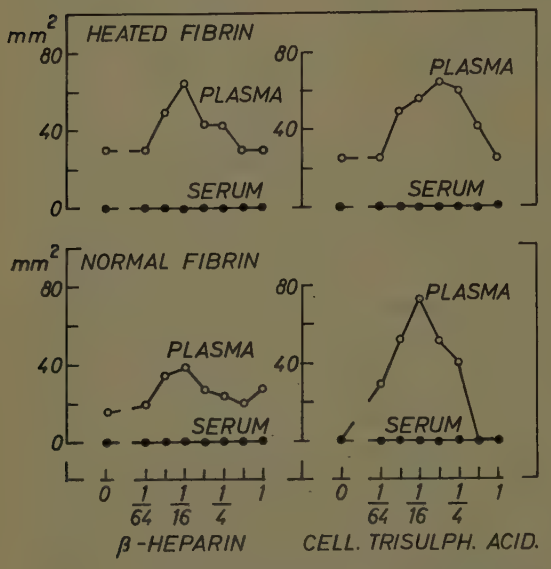


Fig. 2. Fibrinolytic activity of serum and plasma after precipitation in the presence of various concentrations of β-heparin or cellulose trisulphuric acid. *Abscissa:* mg of added compounds per ml of serum or plasma (Logarithmic scale). *Ordinate:* Fibrinolytic activity.

findings in a number of similar attempts to produce fibrinolytic activity in normal human serum and plasma by means of anionic polyelectrolytes: Plasma regularly yields some fibrinolytic activity, whereas serum only occasionally does.

An exception to the rule that plasma gives higher activity than serum was observed in specimens obtained during pregnancy, see Fig. 3. In four cases investigated — two in the second trimester, and two at term — serum produced nearly the same activity as plasma, after a precipitation in the presence of 'purified peptone'. As yet no explanation for this observation can be given.

Heparin-plasma (0.1 mg per ml of blood) has also been examined and compared with oxalate plasma; none of the samples w heparin showed any activity after precipi tion, whether 'purified peptone' was add or not (*cf.* the *inhibitor effect* produced guinea-pig serum by larger concentratio of heparin, Olesen 1959 b).

Spontaneous activity. Various reports dicate that the fibrinolytic activity in sh blood may disappear rapidly. Thus, Fearn & Lackner (1955) demonstrated a 'lab fibrinolytic component' in diluted blood a plasma from healthy persons.

In our normal procedure no special p cautions were taken to conserve any par cularly labile factors. Blood or plasma w often kept for 1 or 2 hours at room tem rature before the assay (or before it w frozen for later use). These circumstan had no demonstrable influence on the ca

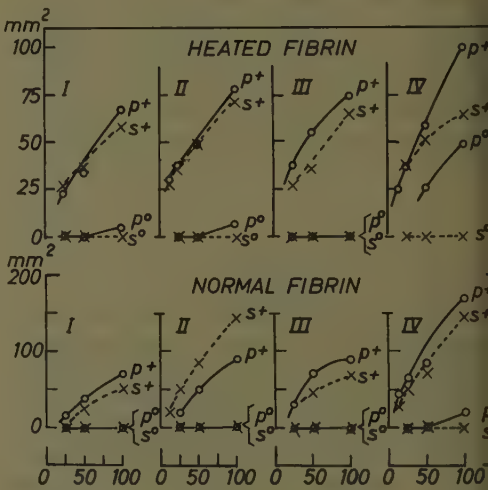


Fig. 3. Blood samples from four pregnant wom The fibrinolytic activity of serum and plasma a precipitation in the absence (S^0, P^0) or in presence (S^+, P^+) of 'purified peptone'. *Absci* Percentage of stock solution. *Ordinate:* Fibr lytic activity.

of plasma to produce fibrinolytic activity
er precipitation in the presence of 'pur-
d peptone'. Even plasma which had been
for several hours at room temperature
oduced fibrinolytic activity of the same
gnitude as was obtained in fresh plasma.
is suggests that the activation produced
peptone depends on factors which are
ble at room temperature.

However, 'spontaneous' activity might be
ised by labile agents as suggested by the
ults of the following experiment. From
person eight consecutive blood samples
re taken at 20 minute intervals. The
lated blood was immediately placed in
-water for 5 minutes before centrifugation.
ereafter the plasma was diluted with ice-
d water, with or without addition of 'pur-
d peptone', adjusted to pH 5.3 to 5.4,
after a few minutes centrifuged for
aration of the precipitates. These were
solved in buffer at room temperature and
mediately applied to the fibrin plates, so
t this step was accomplished about twenty
nutes after the withdrawal of each
od sample. All of these samples (two are
wn in Fig. 4 A) showed considerable
ontaneous' activity, varying from sample
sample, but all of the same magnitude,
with no apparent relation to any known
ditions (*e. g.* the fasting state of the
or, or the 'stress' represented by re-
ted venepunctures). The use of 'purified
tone' had little or no effect on lytic activ-
on normal fibrin, but it produced some
ease in the activity recorded on heated
in. The plasma left over from this experi-
nt was cooled and placed in the deep-
ze after the individual samples had been
t for 1 to 3 hours at room temperature.

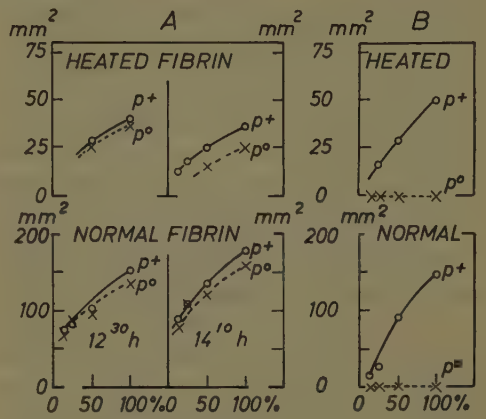


Fig. 4. 'Spontaneous' fibrinolytic activity of human plasma (P^0) compared with 'peptone-induced' activity (P^+), using a 'cold-technique' applied to (A) samples of fresh plasma, or (B) plasma one day old (for further details see text). *Abscissa:* Percentage of stock solution. *Ordinate:* Fibrinolytic activity.

The following day this pooled plasma was again tested in the same way (Fig. 4 B). The 'spontaneous' activity of the fresh plasma had now completely disappeared, whereas the activity produced by peptone was of the same order as the day before (the results were recorded on fibrin plates prepared from the same batch of fibrinogen as used the day before). Precipitation of the pooled plasma at room temperature produced the same result as in ice-water.

DISCUSSION

The experiments presented here demonstrate that fibrinolytic activity can be induced by subjecting normal human *plasma,* but usually *not serum,* to an isoelectric precipitation in the presence of peptone or acid polysaccharides. From analogy with the corresponding reaction in *guinea-pig serum*

(Olesen 1959 a,b, c) this result is considered to indicate that human plasma contains a plasminogen activator system which is similar to that in guinea-pig serum, and is activated by the same procedures. The effect of peptone or of acid polysaccharides in inducing fibrinolytic activity differs from the activating effects produced by streptokinase, tissue activator, or urine activator, since the latter compounds produce activation by simple addition to a system containing the necessary precursor(s) (plasminogen — and in the case of streptokinase also 'proactivator').

The fact that a 'cold technique' revealed 'spontaneous' activity of isoelectrically precipitated human plasma-euglobulin of the same magnitude as the activity produced in the presence of peptone (Fig. 4 A) is consistent with the hypothesis that in either case the same 'activator system' is involved in the formation of fibrinolytic activity (*cf.* the similar conclusion with reference to guinea-pig serum, Olesen 1959 a).

The observation (Fig. 4 B) that the 'spontaneous' fibrinolytic activity had disappeared from plasma after storage while the peptone-induced activity remained unaffected is no contradiction to this concept. The activating agent causing 'spontaneous' activity could exist in a labile form, which during storage combines with other plasma constituents to give a more stable, probably inactive, complex, from which it is no longer released by simple precipitation, but still can be reactivated by the interactions produced by peptone or acid polysaccharides.

The results in this paper indicate that the 'activator system' responsible for the activity produced by peptone or acid polysaccharides is in general considerably less potent human serum than in human plasma. T condition corresponds with the observat by Halse (1948) that heparin could ind lysis of fibrin clots submerged in hu plasma or serum, but far more pronoun in the plasma. Halse assumed that a factor' necessary for the activation of fi nolysis by heparin was present in hu plasma, but lacking in serum. Although experiments cannot be directly compare seems probable that Halse's 'co-factor' responds to our 'activator system'; especi because heparin is one of the acid p saccharides which exert a typical fibrinoly inducing effect in guinea-pig serum (Ol 1959 b).

The difference between serum and pla could be due to adsorption of the active tor on fibrin when the blood is allo to clot. In support of this view, it was served during the present work that fibr lytic activity could be eluated from hu blood clots by means of a buffered solu of commercial peptone. No demonstr activity was obtained when buffered sa was used instead of the peptone solution.

The demonstration that human pla contains a precursor system that m possible the formation of fibrinolytic acti by a precipitation in the presence of pep or acid polysaccharides suggests that 'activator system' could be involved in control of fibrinolysis in the human or ism.

SUMMARY

Fibrinolytic activity can normally be duced in human plasma by an isoele precipitation in the presence of pepton

polysaccharides. Human serum similarly
ed in general gives much less or no
ity. The process of activation in human
na appears similar to that occurring in
ea-pig serum. It depends on an 'activator
m' different from the 'proactivator
m' involved in the activation of fibrino-
by streptokinase.

me observations suggest that the same
vator system' can be responsible for the
rrence of 'spontaneous' fibrinolytic
ity in isoelectrically precipitated human
na.

ne 'activator system' under consideration
l participate in the physiological control
brinolysis.

ACKNOWLEDGMENTS

is investigation was aided by grants to Dr. T.
p, Copenhagen, from the National Danish
ciation against Rheumatic Diseases and from
Josiah Macy, Jr. Foundation, New York.
ks are due to Miss M. Rimestad for skilful
ical assistance.

REFERENCES

p, T., Galsmar, I. & Volkert, M.: Poly-
accharide sulfuric acids as anticoagulants.
Acta physiol. Scandinav. *8*, 215, 1944.

& Müllertz, S.: The fibrin plate method for
stimating fibrinolytic activity. Arch. Biochem.
0, 346, 1952.

— & Olesen, E. S.: Partial purification and fibrinolytic effect of a factor in peptone. Dan. Med. Bull. *4*, 159, 1957.

Fearnley, G. R. & Lackner, R.: The fibrinolytic activity of normal blood. Brit. J. Haemat. *1*, 189, 1955.

Halse, T.: Fibrinolyse. Eine experimentelle und klinische Studie über die 4. Phase der Blutgerinnung. (Cantor, Freiburg 1948.)

Lassen, M.: Heat denaturation of plasminogen in the fibrin plate method. Acta physiol. Scandinav. *27*, 371, 1952.

Müllertz, S. & Lassen, M.: An activator system in blood indispensable for formation of plasmin by streptokinase. Proc. Soc. Exper. Biol. & Med. *82*, 264, 1953.

Olesen, E. S.: Fibrinolytic activity derived from guinea-pig serum by peptone. Acta physiol. Scandinav. *41*, 187, 1957.

— Peptone activation of a serum fibrinolytic system. Acta pharmacol. et toxicol. *15*, 197, 1959 a.

— Effect of acid polysaccharides on the fibrinolytic system in guinea-pig serum. Acta pharmacol. et toxicol. *15*, 307, 1959 b.

— Effect of anionic polyelectrolytes on the fibrinolytic system of guinea-pig serum. Acta pharmacol. et toxicol. *16*, 38, 1959 c.

Ungar, G., Damgaard, E. & Hummel, F. P.: Activation of profibrinolysin by antigen-antibody reaction and by anaphylactoid agents; its relation to complement. J. Exper. Med. *98*, *291*, 1953.

— & Mist, S. H.: Observations on the release of serum fibrinolysin by specific antigen, peptone, and certain polysaccharides. J. Exper. Med. *90*, 39, 1949.

Scandinav. J. Clin. & Lab. Investigation 416—417, 13, 1961.

ON THE DETERMINATION OF CARBONIC ANHYDRASE IN BIOLOGICAL MATERIAL

By Å. HOLMGÅRD

From the Central Clinical Laboratory, S:t Erik's Sjukhus, Stockholm, Sweden

(Received for publication January 2, 1961)

The activity of carbonic anhydrase can be determined by three methods, manometric, electrometric and colorimetric. The manometric procedure (Roughton & Booth, 1938 and 1946) is considered to be the most reliable but technically difficult. Both the electrometric (Wilbur & Andersson 1948) and colorimetric (Philpot & Philpot 1936 and Roughton & Booth 1946) methods are founded on the change of pH, when H_2CO_3 is formed from CO_2 and H_2O, implying an increase of the acidity. The enzyme activity is then calculated as the inverse value of the number of seconds required to obtain a certain of pH. It may therefore be remarked that the enzyme has to react during a continuous change of pH, which will give a continuous fall of its activity. It may also be observed that some indicators inhibit the enzyme. These disadvantages diminish the reliability of the electronic and colorimetric methods.

In this paper a new modification for determination of carbonic anhydrase activity is presented. The pH of the solution is kept constant during the entire determination. A preliminary note on the method has previously been published (Holmgård 1960). The reaction is performed in an autom titrator. Similar methods have been descri for other enzymes, for instance acetylc inesterase. When CO_2 reacts to H_2CO_3, appropriate quantity of NaOH for m tance of a constant pH is automatically continuously added.

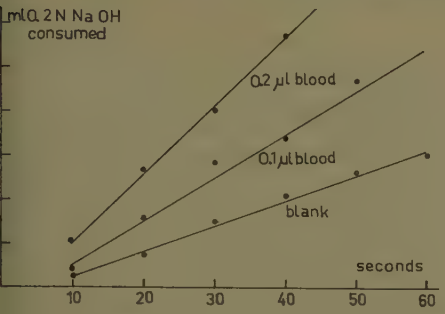
METHOD

Buffer solution: Sodium barbital 4.536 g/1, 8.15.

Substrate: Water, saturated with CO_2.CO_2 is bubbled through the water for one hour.

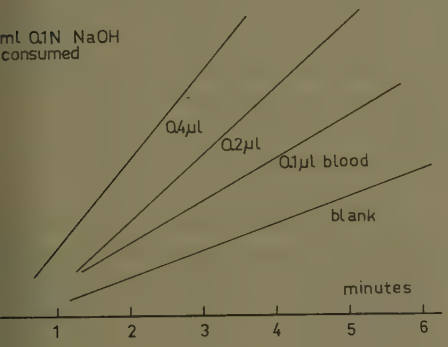
Alkali: 0.2 N NaOH.

In these experiments a Beckman titrator m K was used. Whole blood was used for standar tion of the method, but experiments have also performed on kidney tissue homogenate. The tion is carried out at 0°, since the spontan formation of H_2CO_3 from H_2O and CO_2 is high at higher temperatures. To 5 ml buffer sol containing different amounts of enzyme, 5 ml strate are added. The titrator dial is set at pH The titrator then adds sodium hydroxide acco to the formation of new hydrogen ions. The rea velocity is directly proportional to the amou enzyme present. The reaction takes about one ute. (Fig. 1.) At the same time a blank wi enzyme is run. The velocity is calculated a difference between sample and blank in N consumed per minute.



. The enzyme activity as a function of time.

another series of experiments the CO_2-gas ubbled directly to the buffer in the vessel in the reaction and titration takes place. In vay the reaction can be followed for a longer in Fig. 2 for 6 minutes. The difference be- sample an blank velocities is proportional to mount of enzyme present. (Fig. 3.) It is tant that there is always an excess of CO_2 / gas flow / because an insufficient supply of gives a decrease of the enzyme effect.



2. The enzyme activity as a function of time during continous addition of CO_2.

SUMMARY

A new method for determination of carbonic anhydrase activity is presented. This new method involves titration at a constant pH by use of an automatic titrator. The enzyme activity can be followed continuously.

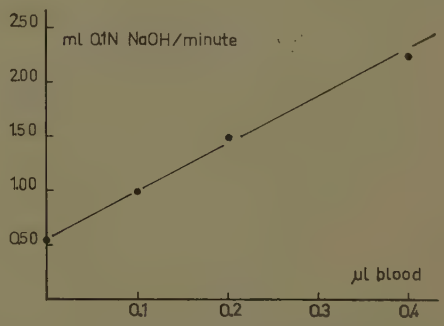


Fig. 3. The enzyme activity as a function of the amount of whole blood.

REFERENCES

Holmgård, Å.: Fourth International Congress on Clinical Chemistry. Edinburgh 1960.

Philpot, F. J. & Philpot, J. L.: A modified colorimetric estimation of carbonic anhydrase. Biochem. J. *30*, 2191, 1936.

Roughton, F. J. W. & Booth, V. H.: The catalytic effect of buffers on the reaction $CO_2 + H_2O \rightarrow H_2CO_3$. Biochem. J. *32*, 2049, 1938.

— & —: Kinetics of carbonic anhydrase. Biochem. J. *40*, 319, 1946.

Wilbur, K. M. & Andersson, N. G.: Electrometric and colorimetric determination of carbonic anhydrase. J. Biol. Chem. *176*, 147, 1948.

Scandinav. J. Clin. & Lab. Investigation 418—428, 13, 1961.

MYORCARDIAL METABOLISM OF GLUCOSE, LACTIC ACID, AMINO ACIDS AND FATTY ACIDS IN HEALTHY HUMAN INDIVIDUALS AT REST AND AT DIFFERENT WORK LOADS [1]

By A. CARLSTEN, B. HALLGREN, R. JAGENBURG, A. SVANBORG AND L. WERKÖ
(with the technical assistance of M. Jagenburg, S. Larsson and I. Myrén)

From the Departments of Clinical Physiology, Medicine and Medical Biochemistry, University of Göteborg, Sweden

(Received for publication January 9, 1961)

The myocardial metabolism is high in relation to the blood flow through the myocardium. This is reflected in the large difference in oxygen content between arterial and coronary sinus blood. The cardiac muscle is richly supplied with oxidative enzymes. Under physiologic conditions the myocardial metabolism is therefore believed to be mainly an aerobic process (Blain, Schafer, Siegel & Bing 1956).

Normally the blood vessels of the myocardium are almost totally separated from blood vessels draining surrounding tissues. A study of the arterio-venous difference in substrates which are assumed not to be noteworthily synthesized or accumulated in the myocardium, will give an estimate of the myocardial utilization of these substances.

In the present study simultaneous cath rization of the coronary sinus and bra artery was made and the arterio-venous erences of different plasma lipids (inclu individual free fatty acids), amino a blood glucose and lactic acid were d mined both at rest and during graded ercise.

MATERIAL

18 subjects tested were healthy, non-hospita volunteers, policemen, aged 24—47 years. B the catheterization, physical examination, ele cardigraphy (leads I, II, III, CR_1, CR_2, CR_4, CR_7, AVR, AVL, AVF) and X-ray of the were performed. The blood lipid level was i cases within normal limits for a Scandin population.

The catheterizations were performed ear the morning when the subjects had been fa for 10—12 hours.

METHODS

Vascular catheterization. The brachial a was catheterized percutaneously using the tech described by Berneus, Carlsten, Holmgren &

[1]) This investigation has been supported by a grant from the Swedish Medical Research Council.

Presented at the Scandinavian Congress of Internal Medicine, Oslo, June 1960.

1954). The polyethylene catheter was in-
ed about 20 cm in a cranial direction. For
theterization of the coronary sinus, catheters
Goodale type were used. To avoid blood
g in the catheters they were flushed with
including one per cent citrate after each
sampling.

was possible to introduce the catheters into
nus in about 75 per cent of the attempted
All had some extra systoles during the in-
tion of the catheter and 3 had a coronary
rhythm for some few minutes after the cath-
vas in place. In one case the manouvre with
theter in the right auricle provoked a short,
ent attack of auricular fibrillation and for
eason further investigations in this case were
oned.

od sampling. Before blood sampling the sub-
studied always rested for at least 30 minutes
the catheter had been introduced.

od from coronary sinus was obtained by
n whereas the arterial blood was allowed to
spontaneously. In each subject only a few
of exercise could be studied due to the large
samples necessary for all the analyses plan-
The total amount of blood taken from each
t was 350—400 ml. When the procedure in-
blood sampling during exercise there was a
cy to syncope on standing after the end of
vestigation.

ysical work 100—600 kpm./min. was perform-
supine position on a bicycle ergometer (Holm-
& Mattsson 1954). Blood samples were ob-
after 15 to 20 minutes on each load.

ravascular pressure was measured with a
gauge manometer (Holmgren 1956). *The*
rate was calculated from an ordinary ECG.

s analysis. Exspired gas was collected in
as bags and samples taken over mercury were
uently analysed for oxygen and carbon di-
according to Haldane and Scholander.

od was analysed for oxygen spectrophoto-
ally (Holmgren & Pernow 1959). Carbon
e tension and pH were measured by a method
uced by Ställberg-Stenhagen (Carlsten &
holm 1960).

Chemical analyses. Lactic acid was determined by the method of Baker & Summerson (1941) as modified by Ström (1949) and glucose according to the anthrone method (Wallenius 1953). The results are given in milligrams per 100 ml of whole blood and are the mean values of duplicate analyses.

Amino nitrogen, amino acids and lipids were determined in citrate plasma. The temperature of the blood was immediately lowered by using chilled citrate solution and by keeping the silicone treated centrifuge tubes in an ice water bath. The centrifugation was carried out in the cold. After oxygenation of the blood the hematocrit value was determined to permit the calculation of the amount of plasma in the citrate blood. Total amino nitrogen was determined in triplicate by a colorimetric ninhydrin method (Jagenburg 1959). The individual amino acids were determined according to Moore, Spackman & Stein (1958) using a picric acid filtrate (Stein & Moore 1954). During the preparation of this filtrate a certain conversion of glutamine to glutamic acid could not be avoided. The 150 cm. column was run at 50° C. A determination of the basic amino acids was not included in the study. Preparation of the total lipid extracts as well as determination of total fat, cholesterol, phospholipids, triglycerides and free fatty acids (FFA) was performed according to earlier described methods used in our laboratory (Svanborg & Svennerholm 1958, Svanborg & Svennerholm, 1961). The FFA were separated from the other plasma lipids by a technique which will be described in detail elsewhere (Hallgren & Svanborg, To be published). The phospholipids were first removed on a column of silicic acid and the FFA were then separated from cholesterol esters and triglycerides by reversed-phase chromatography. With this method there was a recovery of 90—95 per cent of the FFA. Gas chromatography was used for the determination of the composition of the FFA (Hallgren, Stenhagen, Svanborg & Svennerholm 1960).

RESULTS

The physiological characteristica at rest and at different work loads are summarized in Fig. 1. The data are mean values at circulatory steady state.

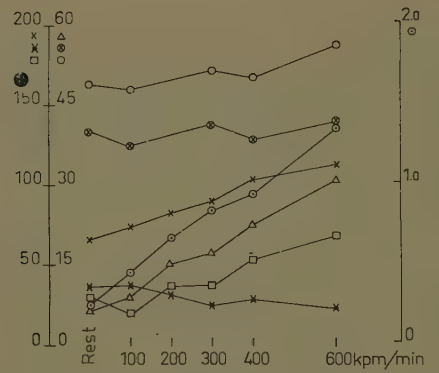


Fig. 1. Physiological characteristica at rest and during exercise. Total body oxygen consumption (⊙) and pulmonary ventilation (Δ) in liter per min. Heart rate per min. (×). Arterial lactic acid concentration in mg per 100 ml of blood (□). Arterial (⊗) and coronary sinus (○) CO_2-tension in mm Hg. Oxygen saturation in coronary sinus blood in per cent (◔).

The work loads were defined as kilopound meter (kpm.) per minute and the biological response to the exercise is characterized by the linear increase in *oxygen intake* (from 0.25 l/min. at rest to 1.4 l/min.) and heart rate (from 65 at rest to 105/min.).

Lactic acid in arterial blood changed only little at work loads up to 400 kpm/min. but started to rise significantly at 600 kpm/min.

pH of arterial blood changed from 7.40 at rest to 7.37 at 600 kpm/min., whereas the carbon dioxide tension fell a little due to the hyperventilation at work. pH of coronary sinus blood was 7.37 at rest and at 600 kpm/min. 7.32.

Oxygen saturation of arterial blood was unchanged during exercise whereas the *saturation of coronary sinus blood* had a tendency to fall. The mean myocardial oxygen extraction was 125 ml/l of blood at w and 120 ml/l at rest[1] ($p = 0.2$).

The oxygen capacity of arterial blood higher during work. At rest the mean v was 17.57 ml 0_2/100 ml, and at 300– kpm/min. 17.91 ml. 0_2/100 ml ($P < 0.0$

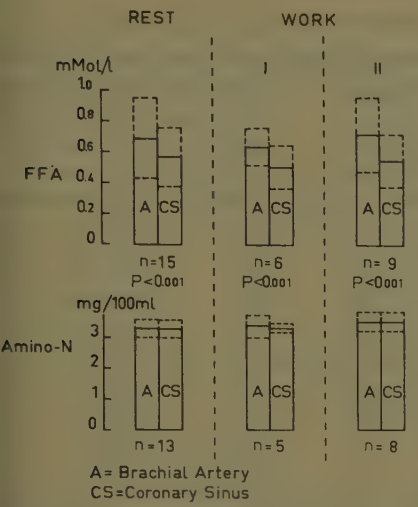
This phenomenon is well-known and sign of hemoconcentration at work.

The oxygen capacity of coronary blood was lower than that of arterial bl The mean value was 16.73 ml 0_2/100 m the coronary sinus blood at rest and 1 ml 0_2/100 ml during 300-600 kpm/ ($P < 0.05$). However, this arterio-ve difference is an artefact and can be w explained by the higher proportion of pla in the blood that has been obtained by tion through the cardiac catheter in the c nary sinus (compare below).

The carbon dioxide tension increased ing the passage of the myocardium from to 49.0 mm Hg at rest and from 39.9 to mm Hg at work.

Studies on lipids, glucose, lactic acid amino acids: Significant coronary art venous difference were found in the l of FFA, glucose, and lactic acid (Tab Figs. 2 and 3) whereas there was no erence in amino nitrogen (Table I Fig. 3), cholesterol, triglycerides and pholipids (Table II) either at rest or d work. The myocardial extraction of gl and FFA was unchanged at different loads but the extraction of lactic aci creased as a result of the increased ar level during work (Table I, Fiigs. 2 an

[1] The p-values were calculated for the ences obtained in each case and not for the ences in mean values.



2. Mean arterial and coronary sinus levels of fatty acids and amino nitrogen during rest and . The dotted lines denote the standard deviation. The P-values are calculated from the individual arterio-venous differences. n denotes the number of observations. Work I = work loads giving al body oxygen consumption ≦ 3.0 times the en consumption at rest; work II = work loads g an oxygen consumption > 3.0 times but 6.0 times the oxygen consumption at rest.

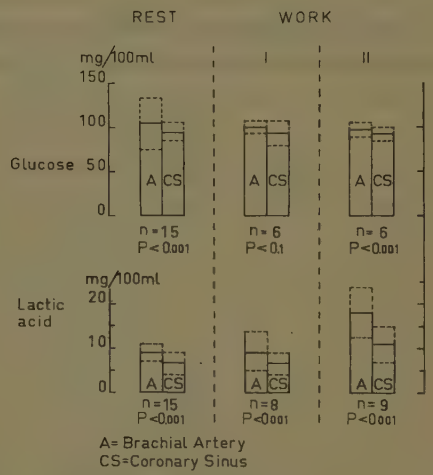


Fig. 3. Mean arterial and coronary sinus levels of glucose and lactic acid during rest and work. The dotted lines denote the standard deviations. The P-values are calculated from the individual arterio-venous differences. n denotes the number of observations. Work I and work II the same as in Fig. 2.

highly significant correlation $r = 0.65$, 29, $P < 0.001$) was found between the rial FFA level and the myocardial ex- ion of FFA (Fig. 4). The arterial level FA was unchanged at the different work s.

he coronary arterio-venous differences in levels of the individual amino acids were rmined in 7 of the volunteers. In all sub- the arterial level of alanine was lower the level in the coronary sinus blood ble III). The mean venous concentration anine (2.97 mg/100 ml) was 19 per cent er than the arterial concentration (2.50 100 ml). On the other hand the level of mic acid was found to be lower in coronary sinus blood. However, during the preparation of the blood sample a formation of glutamic acid from glutamine cannot be prevented. The arterio-venous glutamic acid difference might therefore depend on different concentrations of glutamine in arterial blood and in coronary sinus blood. No significant arterio-venous differences were found in the levels of the other amino acids or in total amino-nitrogen.

A comparison between the composition of the FFA before and after the passage through the myocardium was made in 5 of the subjects. The figures given in Table IV indicate that there were certain changes in the fatty acid composition. The percentage of stearic acid and oleic acid decreased whereas the percentage of linoleic acid increased.

Table I. *The arterial concentration (a) and th myocardial extraction (a-cs) of free fatty acids, glucose, lactic acid and amino nitrogen at rest and at different work loa*

	Subject No.	FFA m Mol/l		Glucose mg/100 ml		Lactic acid mg/100 ml		Amino-N mg/100 ml	
		a	a-cs	a	a-cs	a	a-cs	a	a-
Rest:	1	1.10	0.19	95	–5	9	4	3.68	0.1
	2	0.85	0.05	110	10	8	2	3.66	0.1
	3	0.76	0.10	103	38	9	0	3.59	0.0
	4	0.75	0.06	130	32	12	0	3.40	0.2
	5	0.62	0	102	3	10	4	–	–
	6	0.59	0.25	108	8	12	3	3.42	0.1
	7	0.87	0.37	105	5	7	1	3.11	0.0
	8	0.56	0.13	105	11	11	6	3.57	–0.0
	9	0.67	–0·05	105	11	7	3	2.84	–0.0
	10	0.43	–0.06	103	3	10	5	2.92	–0.0
	11	0.43	0.09	89	5	3	3	–	–
	12	0.58	0.12	109	–2	7	0	3.10	–0.0
	21	1.32	0.56	107	11	12	3	3.40	–0.0
	23	0.32	0.07	99	1	9	4	3.34	–0.1
	29	0.56	0.06	90	5	7	1	2.87	0.0
	Mean	0.69	0.13	104	9	9	3	3.30	0.0
Work: $\frac{\dot{V}O_2 \text{ work}}{\dot{V}O_2 \text{ rest}} \leq 3.0$	4	0.78	0.03	100	–4	15	4	3.92	0.4
	5	0.56	0.19	102	3	12	6	–	–
	7	0.75	0.21	108	3	7	2	3.03	0
	8	0.52	0.12	96	23	10	3	3.59	–0.0
	11	0.50	0.03	91	11	2	2	3.41	–0.0
	12	0.71	0.21	111	4	9	3	2.98	0.0
	Mean	0.64	0.13	101	7	9	3	3.39	0.0
$3.0 < \frac{\dot{V}O_2 \text{ work}}{\dot{V}O_2 \text{ rest}} \leq 6.0$	2	1.20	0.44	100	0	10	3	3.99	0.
	3	1.00	0.33	99	7	13	4	3.72	–0.
	4	0.74	— 0.11	107	9	17	1	3.74	–0.
	5	0.54	0.17	97	4	18	8	–	–
	7	0.68	0.18	106	3	18	6	3.26	0.
	8	0.40	0.11	–	–	21	9	3.79	–0.
	25	0.56	0.13	89	0	26	9	2.97	–0.
	26	0.50	0.06	80	4	11	4	3.35	0.
	27	0.75	0.19	103	9	24	8	3.55	0.
	Mean	0.72	0.17	98	5	18	6	3.54	–0.

Table II. *The arterial concentration (a) and the myocardial extraction (a-cs) otal lipids, cholesterol, phospholipids and triglycerides. Values given in mg/100 ml plasma.*

	Subject No.	Total lipids		Cholesterol				Phospholipids		Triglycerides	
				Free		Total					
		a	a-cs	a	a-cs	a	a-cs	a	a-cs	a	a-cs
:	1	590	−15	83	3	187	29	199	−7	142	0
	2	472	29	78	−3	175	−15	197	19	71	−4
	3	651	56	72	−1	166	−11	244	30	117	5
	4	738	77	75	−3	213	50	251	30	145	−1
	5	652	25	82	6	210	−13	253	10	113	−9
	6	643	13	83	−8	203	−50	207	−3	60	0
	7	760	71	105	−19	292	10	288	80	77	13
	8	416	14	80	38	122	3	163	3	53	12
	9	508	12	40	0	144	−26	190	−1	73	0
	10	642	−10	52	6	189	−13	200	−10	93	10
	11	690	45	78	4	312	6	240	12	33	1
	12	453	−7	40	3	164	18	165	−5	33	2
	21	753	−37	60	−2	202	0	227	−19	127	8
	23	612	8	48	−1	166	−9	216	8	58	0
	29	730	5	63	−7	202	−59	244	10	103	−9
	Mean	621	19	69	1	196	−5	219	10	87	2
k: $\frac{\dot{V}O_2 \text{ work}}{\dot{V}O_2 \text{ rest}} \leq 3.0$	4	777	94	59	11	211	64	252	30	162	41
	5	577	−66	74	−12	189	−19	230	−6	95	−24
	7	730	4	109	−14	278	−2	279	−26	72	10
	8	436	−12	76	13	127	−2	169	−7	46	4
	11	645	−42	76	−3	327	6	240	−3	41	7
	12	438	−3	42	−2	156	−4	156	0	29	2
	Mean	601	−3	73	−1	215	7	221	−2	74	7
$< \frac{\dot{V}O_2 \text{ work}}{\dot{V}O_2 \text{ rest}} \leq 6.0$	2	496	22	92	14	212	18	188	−1	79	−10
	3	608	−5	71	−4	181	8	242	0	118	8
	4	748	23	69	15	148	2	249	7	119	−3
	5	643	39	72	−2	217	−9	260	34	102	−2
	7	702	40	105	−25	287	2	268	30	68	6
	8	441	22	62	4	128	7	149	−36	51	14
	25	563	−13	48	−1	156	1	192	8	74	33
	26	735	9	78	3	290	8	201	4	142	−11
	27	716	−15	56	−4	238	−4	228	−9	120	8
	Mean	628	14	73	0	206	4	220	4	97	5

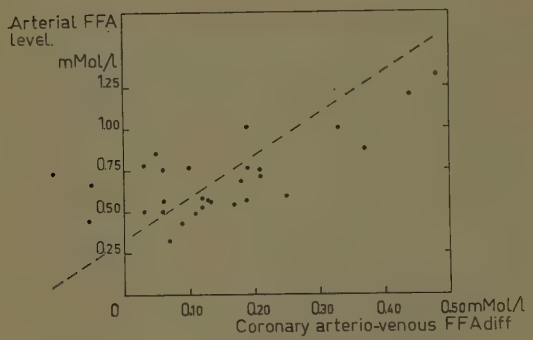


Fig. 4. Myocardial extraction of free acids in relation to the plasma arterial level.

DISCUSSION

The present investigation was concerned with the normal myocardial metabolism and it was therefore necessary to study healt and physically fit subjects. The voluntee were police officers in active service. Res in such subjects may not be quite adequa for comparison with results from hospitaliz patients.

Even fairly small nervous influences m increase the mobilization of FFA (Haml Hickler & Hoskins 1960). In a study like t present it is thus of utmost importance keep the subjects as basal as possible. F this reason the subjects were kept resti 30 minutes before blood sampling. The ox gen consumption determined after this per at rest showed values close to predic values of basal metabolic rate.

Table III. *The arterial (A) and coronary sinus (CS) levels of individual amino ac at rest and at different work loads. Values given in mg. amino acid/100 ml. plasma.*

	Subject 1 Rest		Subject 23 Rest		Subject 29 Rest		Subject 2 Work 200 kpm./min.		Subject 25 Work 600 kpm./min.		Subject 26 Work 600 kpm./min.		Subject 27 Work 600 kpm./min.		Mea
	A	CS	A	CS	A	CS	A	CS	A	CS	A	CS	A	CS	A
Threonine	2.71	3.00	1.65	1.71	1.49	1.46	5.29	5.61	1.41	1.45	1.45	1.55	1.72	2.03	1.74
Serine a)	2.66	2.50	1.72	1.69	1.57	1.69			1.34	1.41	1.33	1.28	1.68	1.66	1.72
Glutamic acid b)	1.84	1.52	3.10	2.24	3.68	3.39	1.64	1.78	3.07	1.01	2.15	1.67	3.87	2.58	2.76
Citrulline			0.59	0.63	0.65	0.64			0.46	0.51	0.41	0.47	0.47	0.56	0.52
Proline	2.20	2.19	3.00	2.94	2.80	2.81	2.81	3.63	2.47	2.31	2.06	2.16	4.63	4.73	2.85
Glycine	1.38	1.39	1.72	1.69	1.66	1.68	2.07	2.13	1.56	1.78	1.53	1.63	1.31	1.56	1.60
Alanine	1.60	1.77	3.62	4.16	1.40	1.68	3.08	3.38	2.74	3.30	2.96	3.05	2.10	3.48	2.50
Cystine	0.73	1.15			1.49	1.29	1.26	1.28	0.66	1.01	1.10	1.30			1.05
Aminobutyric acid	0.20	0.18					0.42	0.36	0.18	0.19	0.16	0.11			0.24
Valine	1.95	1.88	2.80	2.77	2.64	2.68	3.20	3.02	2.40	2.29	2.53	2.36	2.41	2.65	2.56
Methionine	0.40	0.36	0.27	0.25			0.44	0.34	0.35	0.16	0.28	0.29	0.49	0.34	0.37
Isoleucine	0.75	0.68	1.03	0.89	1.11	1.08	0.97	0.67	0.78	0.67	0.91	0.98	0.92	0.78	0.92
Leucine	0.97	1.07	1.72	1.45	1.51	1.37	1.96	1.62	1.21	1.24	1.46	1.64	1.63	1.53	1.49
Tyrosine	0.62	0.59	1.27	1.07	0.76	0.76	1.45	1.08	0.89	0.76	1.20	1.33	1.00	1.03	1.03
Phenylalanine	0.55	0.63	1.04	1.00	1.71	0.77	1.25	0.99	0.74	0.97	0.84	1.12	0.91	0.87	0.86
Total amino nitrogen	3.68	3.53	3.34	3.48	2.87	2.38	3.99	3.76	2.97	3.03	3.35	3.28	3.55	3.54	3.39

a) Includes also glutamine and asparagine: most glutamine, however is destroyed during chr tography.

b) Mainly derived from glutamine, which is partly converted to glutamine acid during the prepar of the sample.

Table IV. *The percentage composition of free fatty acids in arterial plasma and in plasma from the coronary sinus (CS) at rest and at work loads of 600 kpm/min.*

ubject No.	C_{12}	C_{14}	C_{15}	$C_{16:0}$	$C_{16:1}$	C_{17}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	C_{19}	$C_{20:1}$	$C_{20:5}$	$C_{22:1}$	$C_{22:6}$
:														
A	1.5	3.6	0.9	22.5	3.8	2.3	14.1	30.2	7.9	1.2	3.5	0.5	5.1	2.0
C S	1.8	3.9	1.6	21.7	4.1	2.9	11.8	24.2	10.0	1.9	3.0	0.9	9.0	1.0
A	1.5	2.6	0.4	21.1	3.7	1.3	15.0	36.4	6.5	0.4	4.1	0.3	6.2	–
C S	2.6	3.4	0.5	24.7	3.1	1.3	14.9	31.7	6.6	0.6	3.4	–	6.6	–
k:														
A	2.2	4.4	0.6	23.9	5.9	1.6	10.9	34.1	6.6	0.4	2.4	0.8	5.3	0.7
C S	1.9	3.5	0.8	25.1	6.0	1.7	9.8	34.0	7.3	0.5	2.6	0.6	5.0	0.6
A	1.3	4.0	1.0	28.9	5.8	1.2	9.7	38.4	5.2	–	1.7	0.2	1.2	–
C S	1.3	4.0	0.8	31.1	6.1	1.5	8.9	35.5	6.4	–	1.4	–	2.0	–
A	1.1	3.1	0.3	30.3	6.8	2.0	8.3	37.8	6.5	0.3	1.5	0.4	1.1	–
C S	1.0	3.8	1.0	30.4	5.8	1.7	9.6	35.3	7.8	0.3	1.6	0.3	1.0	–

'he solution used for flushing the cathe- contained sodium citrate and not heparin neparin influences the plasma concentra- of fatty acids (French, Morris & Robin- 1958). The amount of sodium citrate en was small and judged to have no meta- c effect. The coronary blood flow was not ermined in the present study as available hods do not give figures for the total ocardial blood flow but only an approx- te value of the blood flow per unit heart ght.

Vhen comparing arterial and venous centration of different substances, any nges in the red cell and plasma volumes st be considered. The swelling of the red s in venous blood raises the hematocrit ut 8 per cent (White, Handler, Smith & tten 1959). In the present study this rce of error was eliminated as the blood oxygenated before analysis.

The arterial blood and the blood from the coronary sinus were taken with different techniques. The blood from the coronary sinus was taken by suction through a long catheter, whereas the arterial blood was allowed to flow spontaneously. In model experiments, when blood was sucked through the catheters used, a decrease in oxygen capacity of about 4 per cent was demonstrated. This relative increase in plasma volume of blood obtained by suction is due to the lower viscosity of the plasma than that of the red corpuscles. The mean difference in oxygen capacity between arterial blood and blood from the coronary sinus, found in the present investigation, was 3.7 per cent and can thus be wholly explained by the fact that different techniques were used for sampling of arterial blood and blood from the coronary sinus. These observations emphasize the importance of determining the hematocrit value

of each blood sample before calculating the amount of plasma in a mixture of blood and citrate solution as in the present study.

The subjects were studied after fasting over night. In the postabsorptive state the myocardium derived most of its energy requirement from the combustion of FFA and to a lesser degree from the combustion of glucose and lactic acid. These findings are in agreement with the results of earlier investigators (Gordon, Cherkes & Gates 1957, Goodale, Olson & Hackel 1959). The analyses of total fat, cholesterol, phospholipids and triglycerides showed no significant decrease during the myocardial passage. As the triglycerides were calculated from glycerol determinations and the phospholipids from phosphorous analyses, we cannot exclude that there has been a release of ester-bound fatty acids during the myocardial passage from these lipid fractions, as has recently been proposed (Ballard, Danforth, Naegle & Bing 1960). Contrary to the results of Blain *et al.* (1956) no significant decrease in amino nitrogen during the myocardial passage was demonstrated in the present investigation. This discrepancy might be due to both nutritional and methodological differences. The method used in the present study for the determination of amino nitrogen is highly specific and accurate enough to establish even small differences.

The reason for the elevation of the alanine level during the myocardial passage is not settled, but might be due to a transamination process as pyruvic acid increases during work (Johnson & Edwards 1937).

The increased myocardial requirement for oxygen and calories during exercise seems to be mainly covered by increased coronary blood flow. Only to a minor extent was t requirement for oxygen and substrate sat fied by an increased extraction. The extra tion of FFA, glucose and amino nitrog was unchanged during work but there was increased extraction of lactic acid in pr portion to the higher arterial level at wor

In the fasting state the decrease in plasm FFA level during the myocardial passa was found to average 18 per cent. The e traction of individual fatty acids was, ho ever, not uniform. The greatest concentr tion change was found among the C 18 fat acids. A percentage decrease was found f stearic and oleic acid. On the other hand t percentage of linoleic acid increased from 6 to 7.6, which compared with the mean d crease in total FFA level indicates that or very little linoleic acid was extracted. T cardiac extraction of the individual fr fatty acids is thus influenced both by cha length and degree of saturation. Howev the nutritional state influences the metabo behavior of the heart muscle and our findin might therefore be valid only for individua in the same nutritional state.

SUMMARY

The myocardial extraction of differe substrates was studied in 18 fasting, healt non-hospitalized individuals by comparis of the levels in arterial plasma and in plas from coronary sinus. The study includ glucose, lactic acid, total amino nitrogen, fr fatty acids, cholesterol, triglycerides, ph pholipids, individual free amino acids a free fatty acids.

The level of free fatty acids, glucose a lactic acid decreased significantly during t

cardial passage. A positive correlation found between the arterial level of free acids and the myocardial extraction. extraction of glucose, amino nitrogen free fatty acids was not influenced by erent work loads but the extraction of ic acid increased as a result of the in-sed arterial level at work. These findings cate that the higher myocardial need for ries during work, is mainly covered by ncrease in coronary blood flow.

Obvious changes in the composition of the fatty acids were found among the C 18 acids. The percentage of stearic acid oleic acid decreased during the myo-lial passage, whereas there was an in-se in linoleic acid. The rise in the per-age of linoleic acid indicates that no lino-acid was extracted during the myo-lial passage.

Although there was no change in the total no nitrogen during the myocardial pas-e, there was a significant elevation of the ine level. Other changes observed in the no acid levels were not conclusive.

REFERENCES

er, S. B. & Summerson, W. H.: The colori-metric determination of lactic acid in biological material. J. Biol. Chem. *138,* 535, 1941.

ard, F. B., Danforth, W. H., Naegle, S. & Bing, R. J.: Myocardial metabolism of fatty acids. J. Clin. Investigation *39,* 717, 1960.

éus, B., Carlsten, A., Holmgren, A. & Seldin-ger, S. L.: Percutaneous catheterization of peripheral arteries as a method for blood sam-pling. Scandinav. J. Clin. & Lab. Investigation 6, 217, 1954.

, J. M., Schafer, H., Siegel, A. L. & Bing, R. J.: Studies on myocardial metabolism. Am. J. Med. *20,* 820, 1956.

Carlsten, A. & Söderholm, B.: Carbon dioxide tension and pH of lymph and arterial blood in anaesthetized dogs. Acta physiol. Scandinav. *48, 29,* 1960.

French, J. E., Morris, B. & Robinson, D. S.: Removal of lipids from the blood stream. Brit. Med. Bull. *14,* 234, 1958.

Goodale, W. T., Olson, R. E. & Hackel, D. B.: The effects of fasting and diabetes mellitus on myocardial metabolism in man. Am. J. Med. *27,* 212, 1959.

Gordon, R. S., Jr., Cherkes, A. & Gates, H.: Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. J. Clin. Investigation *36,* 810, 1957.

Hallgren, B., Stenhagen, S., Svanborg, A. & Svennerholm, L.: Fatty acid composition of the plasma lipids in normals and diabetics. J. Clin. Investigation *39,* 1424, 1960.

— & Svanborg, A.: Separation of plasma free fatty acids. Scandinav. J. Clin. & Lab. Investigation. In press.

Hamlin, J. T., Hickler, R. B. & Hoskins, R. G.: Free fatty acid mobilization by neuroadrenergic stimulation in man. J. Clin. Investigation *39,* 606, 1960.

Holmgren, A. & Mattsson, K.-H.: A new ergometer with constant work load at varying pedalling rate. Scandinav. J. Clin. & Lab. Investigation *6,* 137, 1954.

— Circulatory changes during muscular work in man. Scandinav. J. Clin. & Lab. Investigation *8,* Suppl. 24, 1956.

— & Pernow, B.: Spectrophotometric measurement of oxygen saturation of blood in the determination of cardiac output. A comparison with the van Slyke method. Scandinav. J. Clin. & Lab. Investigation *11,* 143, 1959.

Jagenburg, O. R.: The urinary excretion of free amino acids and other amino compounds by the human. Scandinav. J. Clin. & Lab. Investigation *11,* Suppl. 43, 1959.

Johnson, R. E: & Edwards, H. T.: Lactate and pyruvate in blood and urine after exercise. J. Biol. Chem. *118,* 427, 1937.

Moore, S., Spackman, D. H. & Stein, W. H.: Chromatography of amino acids on sulfonated polystyrene resins. Anal. Chem. *30,* 1185, 1958.

Stein, W. H. & Moore, S.: The free amino acids of human blood plasma. J. Biol. Chem. *211*, 915, 1954.

Ström, G.: The influence of anoxia on lactate utilization in man after prolonged muscular work. Acta physiol. Scandinav. *17*, 440, 1949.

Svanborg, A. & Svennerholm, L.: Determination of unesterified fatty acids in normal human plasma. Clin. chim. Acta *3*, 443, 1958.

— Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in a healt Scandinavian population. Acta med. Scandin 169, 43, 1961.

Wallenius, G.: Some procedures for dextran estin tion in various body fluids. Acta Soc. M upsalien *59*, 69, 1953.

White, A., Handler, P., Smith, E. L. & Stett W.: Principals of Biochemistry. Mc Graw-H Book Company, New York, 2nd edition, 6 1959.

inav. J. Clin. & Lab. Investigation 429—433, 13, 1961.

SPECTROPHOTOMETRIC STUDIES ON DENATURED GLOBIN HEMICHROME

By I. FALBE-HANSEN

From The Finsen Laboratory, Finseninstitutet and Radiumstationen, Copenhagen, Denmark

(Received for publication January 9, 1961)

enatured globin hemichrome (alkaline tinglobin)[1] is formed when oxyhemo- n or hemiglobin are treated with alkali, denatured globin hemochrome is ized, or when hematin is combined with tured globin. In a previous paper (Falbe- sen, 1961) it has been demonstrated denatured globin hemichrome forms part ne alkali-resistant residue obtained by ordinary alkali denaturation methods for rmination of fetal hemoglobin (HbF). error thus introduced is probably of no rtance at high concentrations of HbF, at low concentrations it makes an exact rmination of HbF impossible.

ne literature on denatured glomin hemi- chrome shows many discrepancies as concerns the spectrophotometric findings. It has been the purpose of the present investigation to study some of the problems associated with the conversion of oxyhemoglobin into denatured globin hemichrome by denaturation with alkali.

MATERIALS AND METHODS

Oxalated blood from normal human adults was obtained by venipuncture. The red blood cells were washed three times with saline, and hemolysates purified with $Al(OH)_3$ were prepared as previously described (Falbe-Hansen, 1961). Oxygen was led to the solutions in order to ensure complete conversion into oxyhemoglobin. 200 μl of a solution containing approximately 1 g oxyhemoglobin per 100 ml were added to 16 ml of $^N/_{12}$ NaOH in a 50 ml volumetric flask. After a previously fixed interval of time the denaturation was stopped by adding 1.33 ml 1 N HCl and the pH of the solution was immediately adjusted with 10 ml of a $^M/_6$ phosphate buffer to one of the following values: pH 7.2, pH 7.9, pH 9.4, pH 10.3, or pH 11.3. The volumetric flask was then filled to the mark with distilled water. The spectrophotometric readings were carried out with a Zeiss PMQ II spectrophotometer and a cuvette of 40 mm depth was used.

[1] In this paper the nomenclature suggested by berg & Legge (1949, p. 209) has been used ghout. The prefixes "hemo" and "hemi" de- respectively, ferrous and ferric compounds. "i" or "o" will, however, only be italicized the state of oxidation of the iron is of im- nce, not in words used as generic terms. The ously used terms "hemochromogen" and "pa- matin" have been substituted by "hemochrome" "hemichrome", and in place of "methemoglo- the name "hemiglobin" is used.

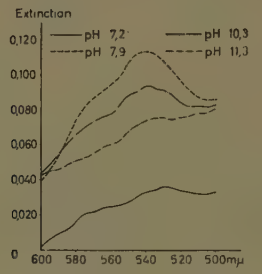


Fig. 1. Spectral absorption curves in the visible region of denatured globin hemichrome at various pH. Denaturation time 1 minute. Some precipitation has occurred in the solution at pH 7.2 and the solution has been filtered.

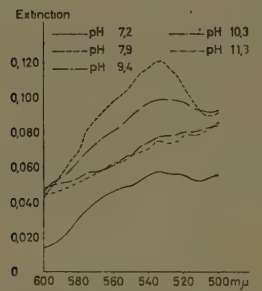


Fig. 2. Spectral absorption curves in the visible region of denatured globin hemichrome at various pH. Denaturation time 2 hours. Some precipitation has occurred in the solution at pH 7.2 and the solution has been filtered.

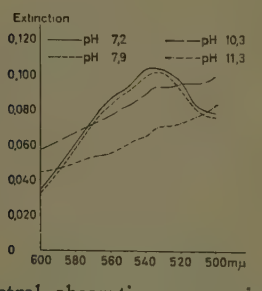


Fig. 3. Spectral absorption curves in the visible region of denatured globin hemichrome at various pH. Denaturation time 18 hours.

RESULTS

In order to get data which might be c
pared quantitatively, solutions of eq
strength of denatured globin hemichro
were prepared from the same hemolys
The denaturation times chosen were 1 m
ute, 2 hours and 18 hours. In Figs. 1, 2 an
are depicted the spectral absorption cur
in the visible region of these solutions
various pH. It must be emphasized that
spectra at pH 7.2 after denaturation
1 minute and 2 hours cannot be compa
directly with the other curves, as some pr
pitation occurred in these solutions. Af
filtration, however, they remained clear.
precipitation took place in the solution at
7.2 after denaturation for 18 hours.

A distinct absorption maximum at ab
540 mμ is found in all solutions at pH
regardless of denaturation time. This ma
mum gradually disappears in more alka
solutions. In the 1 minute denaturation c
ves it is still clearly discernible at pH 1
but after denaturation for 2 hours the
had to be raised to 9.4 in order to obtai
similar shape of the absorption curve.

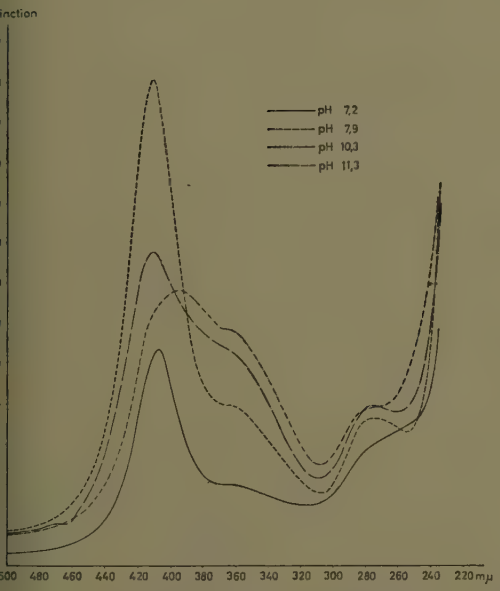
Figs. 4, 5 and 6 show the spectral abso
tion curves in the near ultraviolet and ult
violet regions. At pH 7.9 a strong So
band is present which diminishes with
creasing denaturation time, whereas the w
band at 350—360 mμ becomes more p
nounced. At more alkaline pH the So
band is less sharp and the whole spectr
seems more flattened.

It is noteworthy that the absorption cu
at pH 7.2 after denaturation for 18 hour
identical with the curve at pH 7.9 both
shape and strength of absorbance. The
sence of precipitation may be explained

hift of the isoelectric point of globin as aturation proceeds (Vars & Boxer, 2).

n the ordinary alkali denaturation meth- for determination of HbF, the concen- tion of this compound is estimated by asuring the extinction at 540 mμ. As part the alkali-resistant residue consists of atured globin hemichrome, measurements e been carried out in order to determine v great a fall in absorbance is brought ut by the conversion of oxyhemoglobin denatured globin hemichrome.

Solutions of denatured globin hemichrome e prepared as previously described. De- uration was carried out for 1 minute and



. 4. Spectral absorption curves in the near ultra- et and ultraviolet regions of denatured globin ichrome at various pH. Denaturation time 1 ute. Some precipitation has occurred in the tion at pH 7.2 and the solution has been filtered.

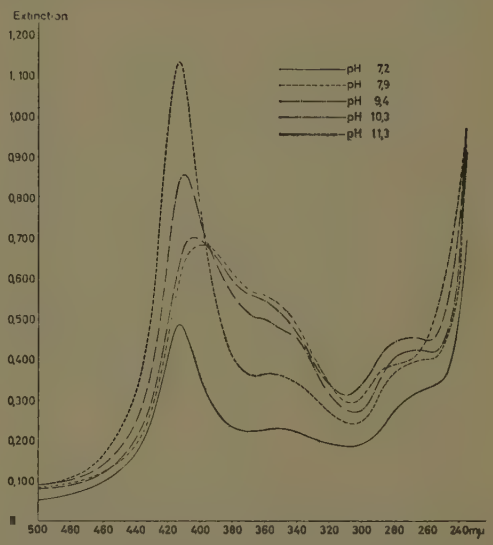


Fig. 5. Spectral absorption curves in the near ultraviolet and ultraviolet regions of denatured globin hemichrome at various pH. Denaturation time 2 hours. Some precipitation has occurred in the solution at pH 7.2 and the solution has been filtered.

pH fixed at 7.9. These conditions were chosen in order to get a close approximation to the conditions under which denatured globin hemichrome is formed when an alkali denaturation method like the one of Singer, Chernoff & Singer (1951) is used. Readings were made at 540 mμ of these solutions and of oxyhemoglobin solutions of the same strength.

In Table I the readings are given of 10 solutions of denatured globin hemichrome prepared from the same hemolysate. The ratio E_{540} oxyhemoglobin/E_{540} denatured globin hemichrome varies from 1.32 to 1.58 with a mean of 1.51.

It is evident from he spectral absorption

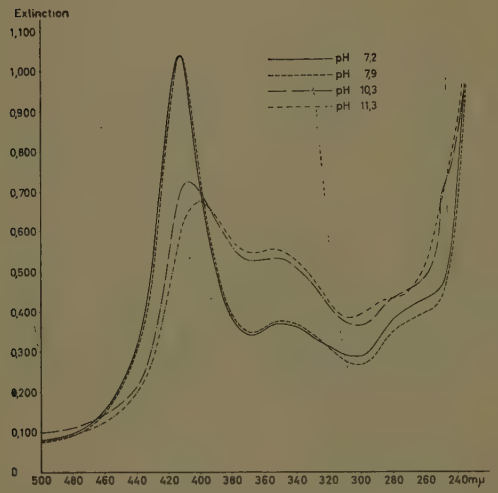


Fig. 6. Spectral absorption curves in the near ultraviolet and ultraviolet regions of denatured globin hemichrome at various pH. Denaturation time 18 hours.

curves that the difference in absorbance at 540 mμ between oxyhemoglobin and denatured globin hemichrome will be found to be even greater at more alkaline pH.

DISCUSSION

The above experiments show that variations in pH lead to changes in the absorption spectrum of denatured globin hemichrome in all regions where measurements have been performed. In accordance with the observations of Austin & Drabkin (1935) and Herzog (1935), it is found that denatured globin hemichrome formed by alkali denaturation of oxyhemoglobin shows no absorption bands in the visible region at alkaline pH. Lemberg & Legge (1949, p. 223) state that a weak absorption band is found in weakly alkaline solutions of denatured globin hemichrome at 558 mμ and a stronger one at 530 mμ, but on further alkalinization the bands disappear. A description of the experimental procedure is lacking.

In the experiments presented here, however, no two-banded absorption spectrum in the visible region was ever found, but it must be mentioned that pyridine hemichrome has two bands similar to those described by Lemberg & Legge (Barron, 1937). Harboe (1959) mentions that the absorption spectrum of denatured globin hemichrome changes with varying pH, but no experimental data are given.

Drabkin & Austin (1935) have found that denatured globin hemichrome formed by autooxidation of denatured globin hemichrome has two distinct bands at 575 and 545 mμ. This absorption curve is practically identical with the one found by Harboe (1959) for solutions containing both oxyhemoglobin and denatured globin he

Table I.

E_{540} oxyhemoglobin/ E_{540} denatured globin hemichrome	
1.48	
1.56	
1.32	
1.56	
1.58	
1.56	mean 1.51
1.53	
1.56	
1.39	
1.56	

The ratio between the extinctions of oxyhemoglobin and denatured globin hemichrome. The extinctions are read at 540 mμ. Ten 1 minute determinations performed on the same hemolysate from a normal adult.

ne. The explanation of this phenomenon be that part of the denatured globin chrome has been renatured into oxy-globin on oxidation (for references to iterature on reversible denaturation of globin, see Lemberg & Legge, 1949, 6).

the present experiments changes in the ption spectrum of denatured globin chrome are furthermore observed with ations in denaturation time. This is in dance with the experiments of Vars & r (1952) on the alkali denaturation of ied globin. These authors find that pro-ive, chemical changes occur in the cule together with a cleavage into units naller molecular size. Considering this, fairly obvious that denatured globin chrome is not one, but several com-ds of varying structure, depending on legree of denaturation of the protein. fact may explain some of the apparantly adictory information on the spectral ption of denatured globin hemichrome. her phenomenon which adds to the con-n is the tendency of hemichromes to nerize (Lemberg & Legge, 1949, 8).

SUMMARY

ectrophotometric investigations have carried out on solutions of denatured n hemichrome prepared by alkali de-ation of oxyhemoglobin. It has been nstrated that considerable changes occur e spectrum of denatured globin hemi-ne with variations in denaturation time and pH. It is suggested that the changes which take place with varying denaturation time are related to progressive alterations in the globin moiety of the molecule as denaturation proceeds. The fact that the formation of denatured globin hemichrome in solutions of oxyhemoglobin causes a decrease in absorbance in the visible region makes the presence of this compound a considerable source of error in the commonly used alkali denaturation methods for determination of HbF.

REFERENCES

Austin, J. H. & Drakin, D. L.: Spectrophotometric studies III. J. Biol. Chem. *112,* 67, 1935.

Barron, E. S. G.: Studies on Biological Oxidations IX. The oxidation-reduction potentials of blood hemin and its hemochromogens. J. Biol. Chem. *121,* 285, 1937.

Drabkin, D. L. & Austin, J. H.: Spectrophotometric studies IV. J. Biol. Chem. *112,* 89, 1935.

Falbe-Hansen, I.: The composition of the alkali-resistant hemoglobin-fraction in blood from normal human adults. Brit. J. Hæmat. *7,* 187, 1961.

Harboe, M.: Studies on the conversion of oxyhemoglobin to alkaline hematin in dilute alkaline solutions. Scandinav. J. Clin. & Lab. Investigation *11,* 138, 1959.

Herzog, A.: Über den Einfluss von Lauge auf Teichmannsches Hämin, Hämatoprosthetin und Hämoglobin. Biochem. Ztschr. *280,* 156, 1935.

Lemberg, R. & Legge, J. W.: Hematin Compounds and Bile Pigments. Interscience Publishers, Inc. New York 1949, pp. 178, 209, 223, 256.

Singer, K., Chernoff, A. I. & Singer, L.: Studies on abnormal hemoglobins. Blood *6,* 413, 1951.

Vars, H. M. & Boxer, G. E.: Modified globin. Chemical changes in human globin produced by alkaline modification. J. Lab. & Clin. Med. *39,* 743, 1952.

Scandinav. J. Clin. & Lab. Investigation 434—438, 13, 1961.

INFLUENCE OF CHOLESTERYL ESTERS FROM HUM SERUM ON SERUM CHOLESTEROL DETERMINATIO WITH THE MODIFIED THEORELL METHOD

By P. BJÖRNTORP and K. CRAMÉR

From the First Medical Service, Sahlgrenska Sjukhuset, and Department of Clinical Chemistry University of Göteborg, Sweden

(Received for publication January 18, 1961)

A previous paper (Cramér & Isaksson, 1959) on the Theorell method for the determination of total serum cholesterol (Theorell, 1926, 1930, Theorell & Widström, 1931) demonstrated that this method, slightly modified, gives accurate values. The total serum cholesterol values were found to be 4.6 per cent higher than the corresponding determinations according to the method of Sperry & Webb (1950), performed on the same extract from 153 sera over the range of 100—600 mg/100 ml.

Esterified cholesterol generally gives stronger color in the Liebermann—Burchard reaction than the equivalent amount of free cholesterol. It was therefore probable that the 4.6 per cent higher values with the Theorell method were, at least partly, due to the unhydrolysed cholesteryl esters. Cramér & Isaksson (1959) did not find any differences in the color-developing properties of free cholesterol and cholesteryl stearate. However, in human serum, cholesterol is mainly esterified with unsaturated fatty acids. It was therefore important to test cholesteryl esters isolated from human serum.

Sperry & Brand (1955) observed that color-developing property of cholestero the Liebermann—Burchard reaction creased after prolonged standing, when The stability under dryness and heat on steam-bath of free cholesterol and se cholesteryl esters was therefore tested.

The purpose of the present investiga was:

1. To determine the chromogenity human serum cholesteryl esters in the m fied Theorell method.

2. To study the stability under dry and heat of free cholesterol and of hu serum cholesteryl esters in the same met

MATERIAL

Cholesterol (BDH) was crystallized twice 99.5 per cent ethanol or ethyl acetate and dri *vacuo* at 80° C for 4 hours or in a drying s overnight at 50° C. This substance had a m point of 148° C and an infra-red spectrogra agreement with that found by Johnson, Idler, loche & Baumann (1953). No water of cryst

as present when the drying was carried out
circumstances described. Cholesterol stan-
in chloroform prepared in the described way
t change their extinction in the reaction in
n up to at least two months.
um cholesteryl esters from 18 subjects with
l or elevated serum cholesterol levels were
d by silicic acid chromatography according
rsch & Ahrens (1958). The cholesteryl ester
ns were not contaminated with glycerides,
ed by analysis for glyceride-glycerol (Carls-
Wadström, 1959), or free cholesterol, as
by paper chromatography (Michalec, 1956).
than 50 per cent of the fatty acids were
saturated, with linoleic acid (about 40 per
and arachidonic acid (about 5 per cent) as
ain constituents, determined by the alkali
ization technique of Holman & Hayes
).
e cholesteryl esters were eluted from the sili-
id columns with a 1:99 mixture of diethyl-
petroleumether, boiling point 40—50° C, and
rated to dryness at room temperature in nitro-
tmosphere. The determinations were per-
d on chloroform solutions of this extract
n average content of 200 mg per ml.

EXPERIMENTAL AND RESULTS

olesterol determinations on cholesteryl
s, isolated from 18 human sera, were

le I. *Determination of cholesterol in n serum cholesteryl esters according to nethod of Theorell and the method of Sperry & Webb (1950).*

	Sperry & Webb	Modified Theorell
Mean of pecimens (μg)	206.5	211.6
ence (μg)	5.2 ± 13.2	
ntage difference	2.5 ± 6.3	

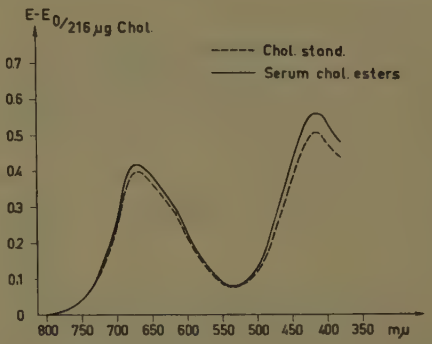


Fig. 1. Absorption curves in the Theorell method for cholesterol and human serum cholesteryl esters.

performed according to the Theorell method and the method of Sperry & Webb (1950). The determinations were made in duplicate or triplicate. The results are given in Table I.

It is seen from the table that the Theorell method gives, on an average, 2.5 per cent higher values than the Sperry & Webb method, when calculated on a molar basis. The dispersion of the differences is considerable, but the difference between the means is statistically significant.

The absorption curves in the Theorell method for cholesterol and the serum cholesteryl esters from one subject were recorded in a Zeiss RPQ 20 A self-recording spectrophotometer. The curves were identical and are presented in Fig. 1.

The test of stability was performed in the following way: 1 ml portions of a standard cholesterol solution in chloroform (285 μg/ml) were added to four Hagedorn tubes and 0.5 ml standard solution + 0.5 ml chloroform to another four tubes. Two tubes from each series were subjected to the color reaction without previous evaporation of the chloroform. The chloroform in the remaining

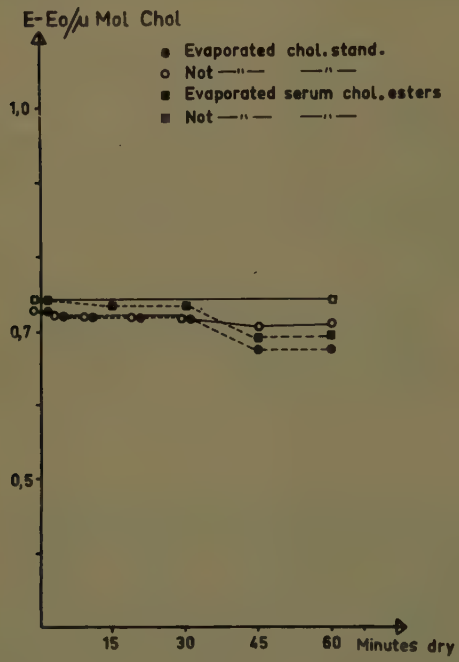


Fig. 2. The color-developing properties of cholesterol and human serum cholesteryl esters in the Theorell method after different periods of dryness on the steam-bath.

tubes was evaporated on the steam bath until cholesterol crystals were visible on the bottom, and then left for an additional period of time on the steam bath before the color reaction was performed. The time of exposure to heat and dryness after the evaporation of the chloroform varied between 5 and 60 minutes.

The same series of experiments was performed with cholesterol dissolved in ethanol: acetone, 1:1, and with human serum cholesteryl esters dissolved in chloroform. The results are seen in Fig. 2.

From the results in Fig. 2 it is seen that cholesterol and human serum cholesteryl esters can be heated on a steam-bath 30 minutes after evaporation of the solv without introducing any error into the de minations. The extinction diminishes a more than 30 minutes' heating.

DISCUSSION

The Liebermann—Burchard reaction its different variations has earlier b applied to purified preparations of ch esteryl esters, mainly acetate or stear Most authors have found these esters to g higher extinctions than the equival amount of free cholesterol. When the action is used for quantitative purposes human serum we must, however, test cholesteryl esters that are actually pres in highest concentration in serum or iso native serum cholesteryl esters.

Human serum cholesteryl esters gave this study, on an average, 2.5 per cent hig molar extinctions than free cholesterol v the method in question as compared with method described by Sperry & Webb (195 Cramér & Isaksson (1959) demonstra that the modified Theorell method gave, an average, 4.6 per cent higher values t the Sperry & Webb method, when deter nations were performed on total serum ch sterol. The last-mentioned difference thus be partly explained by the differenc chromogenity of the cholesteryl esters free cholesterol in the modified Theo method.

The small difference between the m extinctions of free and esterified cholest is somewhat surprising. Sperry & Br (1943) and Kenny (1952) found that w the Liebermann—Burchard reaction carried out in chloroform, as is the cas

present method, there was a considerably er absorbency for cholesteryl esters than free cholesterol. Also in acetic acid the ermann—Burchard reaction gives higher es for cholesteryl esters than for chol- rol, as recently described by Billimoria ames (1960). The present method in- es an 80:1 proportion between acetic anhydride and sulphuric acid with a r maximum at 660 mμ. Most other Lie- man—Burchard modifications use the portion 20:1 with a reading at 620 mμ. se changes may account for the different lts.

he Theorell method is not specific for esterol and its esters. Chenodesoxycholic in serum may influence he determina- s (Isaksson, 1954) and there are also r substances which might disturb the lts (Svanborg & Svennerholm, 1961). vever, the error from these substances is than 2 per cent in subjects where there no signs of biliary obstruction, as de- ed by Björntorp (1960). The disturb- caused by these non-cholesterol sub- ces might explain the rest of the 4.6 cent higher values for total serum chole- ol obtained with the Theorell methods as pared with the Sperry & Webb method.

holesterol and human serum cholesteryl rs left dry on the steam-bath are stable at least 30 minutes as regards their color- eloping properties in the modified Theo- method. After this time, however, the nction in the color reaction decreases. s means that the evaporation must be ormed carefully and that a standard must reated in the same way as the unknown ple for the purpose of control of this or.

SUMMARY

1. The color-developing properties of human serum cholesteryl esters were compared in the modified Theorell method and in the method of Sperry & Webb (1950). The first-mentioned method was found to give, on an average, 2.5 per cent higher values.

2. The stability of cholesterol and human serum cholesteryl esters when dried on the steam-bath in the modified Theorell method was investigated with regard to their chromogenity. After 30 minutes of dryness the color produced had diminished. To control this factor it is necessary to run a parallel standard through the procedure.

REFERENCES

Billimoria, J. D. & James, D. C. O.: Two improved methods for serum cholesterol estimation. Clin. chim. Acta *5*, 644, 1960.

Björntorp, P.: Polyunsaturated fatty acids in man. Scandinav. J. Clin. & Lab. Investigation *12*, Suppl. 52, 1960.

Carlsson, L. A. & Wadström, L. B.: Determination of glycerides in blood serum. Clin. chim. Acta *4*, 197, 1959.

Cramér, K. & Isaksson, B.: An evaluation of the Theorell method for the determination of total serum cholesterol. Scandinav. J. Clin. & Lab. Investigation *11*, 213, 1959.

Hirsch, J. & Ahrens, E. H., Jr.: The separation of complex lipide mixtures by the use of silicic acid chromatography. J. Biol. Chem. *233*, 311, 1958.

Holman, R. T. & Hayes, H.: Determination of polyunsaturated fatty acids in lipides of plasma and tissues. Anal. Chem. *30*, 1422, 1958.

Isaksson, B.: A method for spectrophotometric determination of chenodesoxycholic acid in bile. Acta chem. Scandinav. *8*, 889, 1954.

Johnson, D. R., Idler, D. R., Meloche, V. W. & Baumann, C. A.: Infrared spectra of some cholesterol derivatives. J. Am. Chem. Soc. *75*, 52, 1953.

Kenny, A. P.: The determination of cholesterol by the Liebermann-Burchard reaction. Biochem. J. *52,* 611, 1952.

Michalec, Č.: The nature of cholesterol esters in human blood serum. Biochim. biophys. Acta *19,* 187, 1956.

Sperry, W. M. & Brand, F. C.: The colorimetric detemination of cholesterol. J. Biol. Chem. *150,* 315, 1943.

— & Brand, F. C.: The determination of total lipids in blood serum. J. Biol. Chem. *213,* 69, 1955.

— & Webb, M.: A revision of the Schoenheimer -Sperry method for cholesterol determinati J. Biol. Chem. *187,* 97, 1950.

Svanborg, A. & Svennerholm, L.: Plasma to lipid, cholesterol, triglycerides, phospholip and free fatty acids in a healthy Scandinav population. Acta med. Scandinav. *169,* 43, 19

Theorell, A. H. T.: Über quantitative Bestimmu der Lipoide der aus Pferdeplasma ausgesalt Eiweisskörper. Biochem. Ztschr. *175,* 297, 19

Theorell, H. & Widström, G.: Zur Methodik Lipoidanalysen im Blut unter besonderer rücksichtigung des Gesamtcholesterins. Ztsc f. d. ges. exper. Med. *75,* 699, 1931.

inav. J. Clin. & Lab. Investigation 439—441, 13, 1961.

PERCUTANEOUS TECHNIQUE FOR TRANSSEPTAL LEFT HEART CATHETERIZATION *VIA* THE RIGHT FEMORAL VEIN

By S. BEVEGÅRD, B. JONSSON and I. KARLÖF

From the Laboratory of Clinical Physiology Thoracic Clinics, Karolinska Sjukhuset, Stockholm 60, Sweden

(Received for publication January 21, 1961)

theterization of the left side of the heart become an important adjunct to right catheterization. The transseptal approach, described by Ross (1959) and Cope (1959), seems to be the best method currently available. Provided the technique is satisfactory, no change in hemodynamics occurs and the risk is probably small. The approach from the saphenous vein, using the instrument described by Ross (1959), is somewhat unsatisfactory for studies during exercise with both legs. A method permitting introduction of an instrument from the right internal jugular vein was therefore devised (Bevegård, Carlens, Jonsson and Karlöf, 1960). A percutaneous technique should also make the approach from below more suitable for studies during leg exercise. Such a technique for transseptal left heart catheterization has been described by Cope (1959).

We have found the Seldinger percutaneous technique (1953) to be very satisfactory for catheterization of arteries and veins. When puncturing the atrial septum, it is essential for the catheter to be able to move very easily and without much friction. This permits resistance to be felt when the tip of the catheter is pushed against the septum, and "palpation" of the *fossa ovalis*. Therefore we use a teflon catheter. Continous recording of pressure should be done at the time of septal puncture. Once the needle is in the left atrium, the catheter can be advanced. By so doing, passage of the rather wide catheter into a wrong position, for instance through the pericardium, is avoided. Puncture with the thin needle alone is obviously less traumatic.

METHOD

Equipment required[1].

1) A Seldinger needle for puncture of the femoral vein.
2) A flexible spiral metal guide, described by Seldinger.
3) A teflon catheter.
4) A needle for puncture of the atrial septum.
5) A stylet with a rounded end fitting this needle.

A Seldinger needle, size PE205, is used for puncturing the femoral vein. This needle is then

[1] Available from AB. Stille-Werner, Stockholm.

Fig. 1. Needle for septal puncture with the teflon catheter. Distal end of catheter is closely adapted to needle.

replaced by the flexible metal guide (O.D. = 1.40 mm). The teflon catheter is introduced over the metal guide into the femoral vein. The metal guide and needle for septal puncture have the same outer diameter (1.40 mm). The teflon catheter is easily adapted to both by stretching the tip at a temperature of 200° to 300° C and smoothing it with sandpaper.

The needle for septal puncture is 75 cm long (O.D. = 1.40 mm). The tip is sharpened as an ordinary needle. At the last 8 cm from the tip there is a slight bow. The needle has a luer-lock adapter and attached to this a small wing indicating the direction of the bow at the tip (Fig. 1). The stylet fitting this needle has a diameter of 0.60 mm and its tip is rounded.

Procedure.

The teflon catheter is introduced percutaneously into the right femoral vein using Seldinger's technique. After puncture of the vein a few cm below the *ligamentum inguinale* the flexible end of the metal guide is introduced through the needle and pushed up about 10 cm in a cranial direction. The needle is withdrawn and the teflon catheter is pushed on the guide until the tip of the catheter is exactly at the tip of the guide. The tip of the catheter is therefore visible at screening and can be placed in the right atrium. The guide is withdrawn, and the long needle, with the stylet inserted, is passed through the catheter up to the right atrium. As the needle is slightly bent at the tip it gives some resistance when passed through the catheter, but if moved gently and rotated it is not difficult. The tip of the stylet is about 1 cm ahead of the tip of the needle in order to avoid penetration of the wall of the catheter by the sharp needle. With the tip of the needle a few mm from the end of the catheter, the stylet is withdrawn and the needle connec to a manometer through a polyethylene cathe During screening, it is easy to check the posi of the needle. The tip of the catheter is direc cranially, posteriorly and to the left. Thus *fossa ovalis* and its limbus is "palpated". W placed in the *fossa ovalis*, the needle is pushed ward and, with slight presure, the septum is pu tured during continous recording of pressure. W left atrial pressure is obtained, the catheter pushed forward and the needle withdrawn. With metal guide inserted the catheter can also be mo into the left ventricle while screening. It is possible to pass a thin polyethylene catheter (PE 50) through the teflon catheter when its ti in the left atrium. The thin catheter follows blood stream into the ventricle in most ca though less commonly where there is a la atrium and combined mitral stenosis and incon tence. Using a special adapter, pressures can recorded from both catheters simultaneously (2).

RESULTS

Twenty-one patients have been exami with this technique. In two of the first cases in the series, septal puncture was

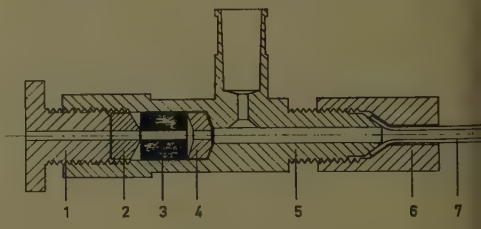


Fig. 2. Diagram of catheter adapter. 1) Metal screw with central opening for polye lene catheter (PE 50). 2 and 4) Metal rings Rubber cuff. 5) 2-way adapter. 6) Screw-on m adapter. 7) Teflon catheter. When the metal sc is tightened, both metal rings compress the ru cuff. This results in an air tight connection permits accurate registration of pressure cu through both the teflon and the polyethy catheters.

essful. It was found that the needle had
trated the catheter a few cm proximal
s tip. Since then, the stylet has been in-
ed into the needle during its passage
ugh the catheter, and no further diffi-
es have been encountered in puncturing
septum.

ressures both in the left atrium and ven-
e were obtained in all 19 cases where the
al puncture was successful. In one case,
ever, the left ventricular pressure could
btained only through a catheter intro-
d from the brachial artery passing the
ic valve into the left ventricle. There was
ght mitral stenosis with a thin regur-
t jet (confirmed at operation), and a
large left atrium. In addition, there was
ild aortic stenosis. In one case of tight
al stenosis, the left ventricle was cathe-
ed from the aorta, and no attempt was
e to pass the mitral valve. In the 17 cases
re the catheter passed through the mitral
e, there was an aortic valvular lesion
e in five and a mitral valvular lesion in
cases, of which four had an associated
ic valvular lesion. Several cases had com-
d mitral valvular disease with tight
osis and a large left atrium.

here were no complications. Most pa-
s did not even feel the puncture. Some
a mild sensation in the chest when the
le was pushed through the septum.

DISCUSSION

he patient can easily perform leg exercise
the catheter inserted by this technique.
eters may be inserted in the pulmonary
brachial arteries. It is also possible to
orm catheterization of the right side with
same catheter used for the left side.

It is important to avoid air embolism when the metal guide is withdrawn. The opening of the catheter is located much higher than the tip of the catheter in the heart. If the patient during this short period does a Valsalva manoeuvre, blood comes out of the catheter. Still better is to place the patient in a slightly tilted position.

SUMMARY

A modification of Cope's (1959) percutaneous technique for transseptal left heart catheterization is described. We have used teflon catheters which have a low friction coefficent. The atrial septum is punctured during continuous recording of pressure through the thin puncture needle.

Both the right and left sides of the heart can be catheterized with this percutaneous technique. Twenty-one patients with valvular lesions in the left side of the heart were examined. In 19 cases, puncture of the atrial septum was successful. Left ventricular pressure was obtained in 17 of 18 cases where an attempt to pass the mitral valve was made. No complications occurred. The patient can easily perform leg exercise with the catheter inserted by this technique.

REFERENCES

Bevegård, S., Carlens, E., Jonsson, B. & Karlöf, I.: A technique for transseptal left heart catheterization via the right external jugular vein. Thorax. *15*, 299, 1960.

Cope, C.: Technique for transseptal catheterization of the left atrium: Preliminary report. J. Thoracic Surg. *37*, 482, 1959.

Ross, J.: Transseptal left heart catheterization. Ann. Surg. *149*, 395, 1959.

Seldinger, S. I.: Catheter replacement of needle in percutaneous arteriography. New technique. Acta radiol. *39*, 368, 1953.

Scandinav. J. Clin. & Lab. Investigation 442—446, 13, 1961.

THE INFLUENCE OF TREATMENT WITH SODIUM SALICYLATE ON THE SERUM GLUTAMIC OXALACETIC TRANSAMINASE ACTIVITY

By AA. DRIVSHOLM[1] and S. MADSEN

From Department 3 and 9 and the Central Clinical Laboratory, Kommunehospitalet, Copenhagen, Denmark

(Received for publication February 3, 1961)

As originally demonstrated by Nydick, Tang, Stollerman, Wroblewski & LaDue (1955) the serum glutamic oxalacetic transaminase(SGO-T) is often increased above the normal range in patients suffering from acute rheumatic fever with active carditis. The same observation was made by Massie & Stahlman (1958) and by Cattaneo, Pelocchino, Pronotti & Rigazio (1958).

Nydick *et al.* (1955) pointed out that an elevated SGO-T is most pronounced in patients suffering from rheumatic fever with cardial insufficiency resulting in stasis of the liver, and mentioned that necrosis of the liver cells might be a contributory cause of the elevated SGO-T. The same authors also mentioned the possibility of a hepatotoxic effect of sodium salicylate, as several of the patients investigated were being treated with this drug. However, a dose of 4—6 g aspirin daily for one week to a group of patients not suffering from rheumatic fever produced no changes in the SGO-T activity. The same result was observed by Massie & Stahlman (1958) who gave a "large dose" of salicylate to one normal patient, to one patient wi severe stasis of the liver, and to seve patients with severe diseases of the liver. the other hand, Manso, Taranta & Nydi (1956) reported an elevated SGO-T in out of 14 children with an aspirin or sodi salicylate intake of 0.6—1 g/15 lbs.[1]/d until symptoms of intoxication were o served. The children, aged 5—15 years, w recovering from rheumatic fever. None the children had active rheumatic fever.

As an elevated SGO-T in patients w rheumatic fever is mentioned as a sign myocarditis (Nydick *et al.*, 1955) and rheumatic fever is often treated with sa cylate, the present investigation was carr out to examine whether long-term treatm with salicylate in patients not suffering fr diseases of the liver or the heart results an increased SGO-T activity.

MATERIAL AND METHODS

The material includes 9 patients, aged 22- years, four women and five men. Five of patients had been admitted to hospital for ac

[1] Present address: Rigshospitalet, Copenhagen.

[1] lb. = 454 gm.

bago, the remaining four for different uncom-
ated fractures.

Before the investigation was carried out the
r function of the patients was examined. Icteric
x, thymol turbidity, alkaline phosphatase, pro-
mbin-time, paper electrophoresis and GO-T of
he serum were done and bromsulphalein reten-
test was carried out. These examinations re-
ed no signs of decreased liver function. Nor
any patients show sign of myocarditis on elec-
ardiography.

During the examination blood samples were
drawn daily, in some patients every third day.
serum was examined for icteric index, thymol
idity, GO-T and for the concentration of sali-
c acid. The icteric index was determined by the
ilengracht method, thymol as extinction, SGO-T
trophotometrically according to Henley & Pol-
's method (1955) and the concentration of sali-
c acid in serum according to Coburn's method
nodified by Faber & Schmidt (1949). The nor-
range of SGO-T was determined to 0.35—1.55
Mol/l/L (95 per cent area). The maximum daily
ation in the SGO-T in 12 patients without
ases of the liver or acute cardiac diseases was
rmined to 0.49 m Mol/l/L (42 observations).

The salicylate given orally as "mixtura natrii
ylatis" 45—90 g (3—6 g sodium salicylate)
y for an average of three weeks (10—33 days),
aim being to produce a concentration of sali-
c acid in serum higher than 20 mg/100 ml as 20
100 ml is given as the lowest value of sympto-
ic effect of salicylic acid in cases or rheumatic
r (Møller, 1958).

To exclude the possibility that uncomplicated
tures and confinement to bed might cause *(per*
an elevated SGO-T, the SGO-T was measured
bedridden patients with uncomplicated fractures
2—4 weeks. In none of the patients was any
O-T elevation observed.

RESULTS

The results are recorded in Table I. One
ient refused further intake of salicylic
l after 10 days. Four of the patients
eloped symptoms of salicylic intoxication, which necessitated a reduction of the daily dose in two of the patients (Nos. 2 and 3) and in another patient (No. 8) prevented any further increase of the initial dose of 3 g sodium salicylate. The intoxication symptoms, nausea and epigastric pain, are indicated by (+), while tinnitus and deafness are indicated by +. None of the patients vomited. The maximum concentration of salicylic acid in serum (43 mg/100 ml) was observed in a patient who showed no symptoms of salicylic intoxication. In three of the patients (Nos. 3, 7 and 9) a significant SGO-T elevation was observed.

In Figs. 1 and 2 are shown the relation between the salicylate dose, the concentration of salicylic acid in serum and the SGO-T values in one patient with and in one without an SGO-T elevation. The thymol values and the icteric index are not shown in the graphs as no significant increases in these values were observed in any of the patients during the examination.

The investigation thus seem to indicate that treatment which salicylate, in doses used in the treatment of rheumatic fever, can cause a moderate elevation of the SGO-T in patients without diseases of the liver and heart.

DISCUSSION

An examination of a possible effect of salicylic acid intake on SGO-T is of interest because an SGO-T elevation is often used as a sign of active carditis in patient with rheumatic fever.

Manso *et al.* (1956) have previously shown that sodium salicylate in very large doses can effect the liver and Thomsen &

Table I. *The influence of sodium salicylate on SGO-T activity.*

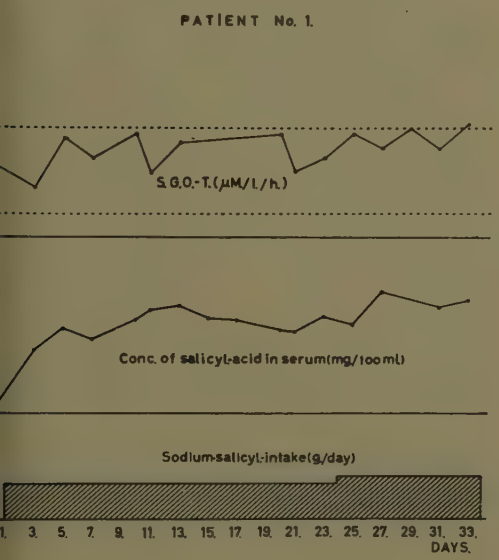
Case	Sex	Age (Years)	Diagnosis	Sodium salicylate intoxication	Duration of sodium salicylate intake (days)	Sodium salicylate intake (g/day)	Maximum concentration of salicylic acid in serum mg/100 ml)	Mean concentration of salicylic acid in serum (mg/100 ml)	Maximum of SGO-T before sodium salicylate intake (units)	Maximum of SGO-T during sodium salicylate intake (units)	Number of elevated SGO-T values during sodium salicylate intake	Maximum of icteric index during sodium salicylate intake	Maximum of thymol turbidity during sodium salicylate intake
1	♂	31	Degeneration of lumbar disc	0	33	6	34.6	26.5	1.05	1.61	0	8	0.05
2	♂	52	Protrusion of lumbar disc	+	22	5	24.8	12.7	1.05	1.40	0	6	0.04
3	♀	56	Degeneration of lumbar disc	(+)	21	4	22.4	16.1	1.26	2.46	5	5	0.08
4	♂	63	Degeneration of lumbar disc	0	21	6	34.0	21.0	1.12	1.19	0	4	0.03
5	♀	69	Degeneration of lumbar disc	0	22	6	29.2	26.0	1.19	1.19	0		0.02
6	♂	22	Fracture of left leg	0	23	6	27.0	18.1	1.19	1.40	0	4	0.02
7	♂	54	Fracture of tarsus	0	21	6	43.0	30.6	1.12	2.88	5	6	0.06
8	♀	58	Fracture of the leg	+	22	3	25.0	20.3	1.33	1.61	0	5	0.09
9	♀	62	Fracture of the femur	(+)	10	6	19.0	13.8	1.40	2.39	4	3	0.04

algård (1958) have found that this can use an SGO-T elevation.

The effect of sodium salicylate intake in erapeutic doses on SGO-T is, as already entioned, examined by Nydick *et al.* 1955), Massie & Stahlman (1958) and anso *et al.* (1956).

The result of our examination is in cordance with Manso *et al.*, 1956. No rect comparison is, however, possible as ey do not specific the concentrations of e salicylic acid in serum. Moreover, they rried out their investigations with children, hereas the present material only comprise lults.

The influence of sodium salicylate on the GO-T is not caused by a direct effect on


PATIENT No. 1.
S.G.O.-T.(μM/l/h.)
Conc. of salicyl-acid in serum(mg/100ml)
Sodium-salicyl-intake(g/day)
1. 3. 5. 7. 9. 11. 13. 15. 17. 19. 21. 23. 25. 27. 29. 31. 33.
DAYS.

g. 1. Relation between the salicylate dose, the ncentration of salicylic acid in serum and the GO-T values in a patient (No. 1) *without* SGO-T vation after salicylate intake. The dotted lines dicate the normal range (95 per cent area) of the SGO-T.

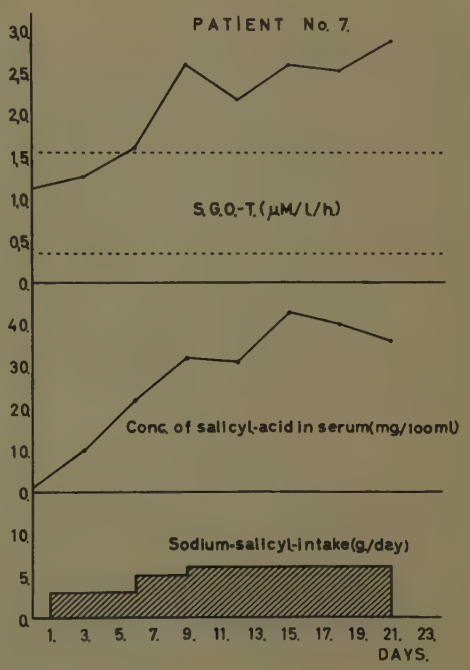


Fig. 2. Relation between the salcylate dose, the concentration of salicylic acid in serum and the SGO-T values in a patient (No. 7) *with* SGO-T elevation after salicylate intake.

the reagents which are used to determine the SGO-T, nor has the substance any influence on the actual spectrophotometric measurements in the concentrations used in this examination.

There seems to be no direct proportion between the SGO-T elevation and the concentration of salicylic acid in serum and the duration of the sodium salicylate intake. It seems reasonable to assume that the sensibility of the liver to salicylic acid is individual. This may explain why some patients develop an SGO-T elevation on a given concentration of salicylic acid in serum, while others do not.

SUMMARY

Nine patients, aged 22—69 years, without any signs of diseases of the liver or the heart, were treated for an average period of three weeks with sodium salicylate in doses of 3—6 g daily in order to investigate whether this treatment may cause an increased SGO-T activity. Three of the patients showed small but significant SGO-T elevations. No certain correlation was observed between the SGO-T elevation and the concentration of salicylic acid in serum.

REFERENCES

Cattaneo, R., Pelocchino, A. M., Prinotti, C. & Rigazio, G.: Changes in Certain Serum Enzymes (Oxalacetic and Pyruvic Transaminase, Lactic Dehydrogenase, and Cholinesterase) in Rheumatic Fever. Reumatismo *100,* 366, 1958.

Faber, V. & Schmidt, K.: Serumsalicylsyrebestemmelse og toxiske Blodforandringer ved intensiv Salicylsyrebehandling. Ugesk. f. læger *111,* 1096, 1949.

Henley, K. S. H. & Pollard, H. M.: A new met for the determination of glutamic oxalacetic glutamic pyruvic transaminase in plasma Lab. & Clin. Med. *46,* 785, 1955.

Manso, C., Taranta, A. & Nydick, I.: Effec aspirin on serum glutamic-oxaloacetic and rum pyruvic-transaminase in children. P Soc. Exper. Biol. & Med. *93,* 84, 1956.

Massie, R. W. & Stahlman, M.: Serum oxaloac transaminase activity in acute rheumatic fe J. Dis. Child. *95,* 469, 1958.

Møller, K. O.: Farmakologi, 5. edition, 564, 1 Nyt Nordisk Forlag, Copenhagen.

Nydick, I., Tang, J., Stollerman, G. H., Wr lewski, F. & LaDue, J. S.: A study of char in serum concentrations of the enzyme, g amic-oxaloacetic transaminase in rheum fever. Circulation *12,* 754, 1955.

— Tang, J., Stollerman, G. H., Wroblewski & LaDue, J. S.: The influence of rheum fever on serum concentrations of the enzy glutamic oxalacetic transaminase. Circula *12,* 795, 1955.

Thomsen, Aa. & Dalgaard, O. Z.: Haemodial in acuta acetylsalicylic acid poisoning. Am Med. *25,* 484, 1958.

Scandinav. J. Clin. & Lab. Investigation 447—461, 13, 1961.

THE ^{131}I-POLYVINYLPYRROLIDONE (^{131}I-PVP)TEST IN GASTROINTESTINAL PROTEIN LOSS[1]

By S. JARNUM

(with the technical assistance of Bodil Møller-Petersen and Lis Hansen)

From the Department of Clinical Biochemistry and Medical Department B, Bispebjerg Hospital, Copenhagen, Denmark

(Received for publication February 1, 1961)

In 1958 Gordon devised a method for preparing a stable and chemically inert ^{131}I-labelled polyvinylpyrrolidone (PVP) for medical use. He suggested that it should be used for investigations on plasma protein metabolism in human subjects, and later (Gordon 1959, Gordon, Bartter & Waldmann 1959) he demonstrated that it was highly useful as a diagnostic tool in *protein-losing gastroenteropathy*. In this condition an abnormally high fraction of ^{131}I will be excreted in the stools after introduction of the labelled substance in the blood stream. In all cases of so-called *"idiopathic hypoproteinemia"* in whom the test was done, it turned out to be abnormal. Thus he solved the year-old mystery concerning the mechanism of the low serum protein concentration in this rare condition. Applying Gordon's ^{131}I-PVP test in other cases of idiopathic hypoproteinemia, a number of authors have reported identical results (Hsia, White & Hugh-Jones 1959, Martini, Strohmeyer & Bünger 1960, Royer 1960, Schwartz & Jarnum 1959, Vesin, Troupel, Acar, Renault, Desbuquois & Cattan 1960, Vesin, Renault & Cattan 1959, Young, Fowler, Barnett & Miles 1959). Furthermore, the ^{131}I-PVP test has demonstrated an abnormal gastrointestinal protein loss in previously well-known diseases associated with hypoproteinemia, *e. g.* gastric carcinoma (Jarnum & Schwartz, 1960), regional enteritis (Steinfeld, Davidson, Gordon & Greene 1960, Vesin, Troupel, Acar, Bismuth, Renault & Cattan 1960), ulcerative colitis (Steinfeld *et al.*, 1960) and sprue (Parkins, 1960).

The present communication reports experience with PVP tests performed in a number of normo- and hypoproteinemic subjects together with an evaluation of PVP as a quantitative test of abnormal gastrointestinal protein leakage.

[1] This work was supported by grants from Statens Videnskabsfond, Kong Christian den X's Fond and Fonden af 21/12 1956 til Lægevidenskabens Fremme.

METHODS

^{131}I-PVP.

Radio-iodinated PVP was supplied by Gordon, U.S.A. Before shipment it was autoclaved and tested for pyrogenicity, and the major part of unreacted iodine had been removed by dialysis. Glucose was added for isotonicity and benzyl alcohol as a preservative. The average molecular weight of the polymere was 30000—40000, and the solution generally contained from 60 to 270 μC per ml on the day of manufacture.

21 different preparations were applied. No further dialysis was undertaken after arrival. In each batch the amount of ^{131}I passing a cellophane[1] bag in 24 hours was determined, and all preparations were applied within 1—3 weeks of manufacture. A weighed amount containing 15—25 μC was given intravenously to the subjects investigated. Ordinarily each preparation was tested in at least one control person with normal serum protein pattern. Blood samples were drawn 15 minutes after the injection and at daily intervals for 3—4 days. Five days (120 hours) after the injection the patients received 1 g carmine *per os*. Stool collection began at once and continued till the stools became red. The total amount of feces was homogenized in a blender. Urine was collected for 5 days.

The amount of radioactivity excreted was measured in a scintillation detector designed for large volumes (500 ml), plasma activity in a well type scintillation counter connected with a Tracerlab 1000— or Super-Scaler. The counting equipment furnished a background value of about 110 counts per minute. Samples of low activity (*e.g.* stools from 22 control subjects) were counted for a period (10—30 minutes) sufficient to ascertain that the proportional standard error of the net count was less than 10 per cent, and in most cases it was appreciably less. In 19 of 22 control subjects the net counting rate of feces was from 50—680 per cent of the background value.

The activity of the samples was compared to that of appropriately prepared standards. The amount of non-dialysable ^{131}I in plasma and excreta was determined after previous dialysis in cellophane bags against running tap water for 3 hours. Preliminary experiments showed that only a slight amount of ^{131}I was removed by further dialysis.

Plasma volume.

In order to estimate the immediate distribution volume of ^{131}I-PVP, a weighed amount of T-1824 was injected intravenously immediately prior to or after the ^{131}I-PVP. The distribution volume of T-1824 15 minutes later was taken as plasma volume x 1.02, allowing for a 2 per cent escape of the dye during mixing time. T-1824 concentration in plasma was determined by a modified extraction method (Jarnum, 1959). A check was made that the optical density of Evans Blue was uninfluenced by the low concentration of PVP in plasma. High concentrations may produce a notable increase in the light absorption of Evans Blue (Allen, Och Roth & Gregersen, 1953).

^{131}I-albumin metabolism.

Albumin metabolism was studied by labelled albumin in most of the hypoproteinemic subjects, as a rule one week after the PVP-test. ^{131}I-albumin was prepared according to the method of Vea Pearson & Hanley (1955). After intravenous injection of a weighed amount of the ^{131}I-albumin solution, radioactivity was determined in daily plasma samples and 24-hour urine specimens. The cumulative fecal ^{131}I-excretion was measured through a 5-day period. Albumin metabolism was calculated after Pearson, Veall & Vetter (1958), or — if quantitative urine collection could be ascertained — after Matthews (1957).

Serum protein was determined by Kjeldahl analysis, serum albumin by paper electrophoresis (Laurell, Laurell & Skoog, 1956).

PATIENTS

A total of 79 patients were studied.

Control subjects.

To estimate the absorption of ^{131}I-PVP from the gastrointestinal tract, 10 patients were given

[1] Membranfilter Gesellschaft, Göttingen, West Germany (thickness: about 90μm).

[1] ½ per cent Evans Blue, Warner-Chilcott U.S.A.

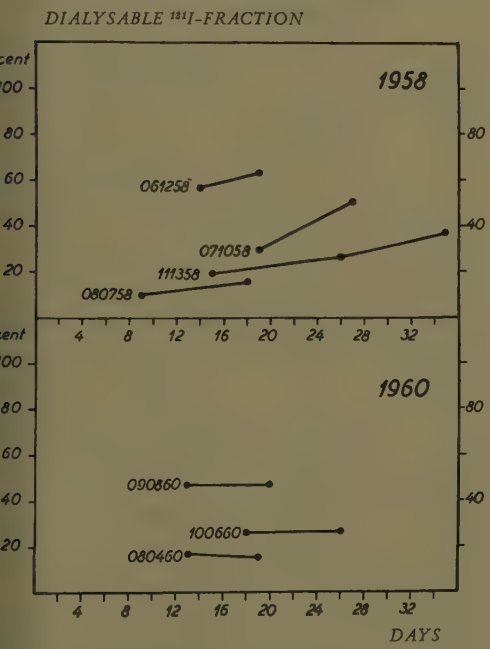
-3 μC *per os*, and the subsequent fecal activity s measured in a 5-day period.

22 patients had ^{131}I-PVP intravenously. They ffered from a variety of diseases (Table I) but had a normal hemoglobin concentration and splayed no signs of hepatic or renal disease. In patients serum albumin concentration was termined and found to be normal.

ypoproteinemic patients.

Patients with hypoproteinemia (notably hypo-buminemia) included 12 patients with "idiopathic poproteinemia" (Table II), 11 cancer patients Table III, which further contains 2 normoprote-emic cancer patients), 8 with chronic diarrhea of rious origin (Table IV), 7 with postgastrectomy ndrome (Table V) and 7 with chronic hepatic sease or nephrotic syndrome (Table VI).

All patients were in a stable condition during the rformance of the ^{131}I-PVP test, which took 5—7 days. Potassium iodide — 1.3 g daily — was given to all patients to prevent thyroid ^{131}I-uptake.



g. 1. Change of dialysable ^{131}I-fraction in ^{131}I-VP preparations of increasing age. *Upper half:* eparations from 1958. *Lower half:* preparations from 1960.

RESULTS

In vitro tests.

In one preparation (090359) almost all activity passed a cellophane membrane. In the remaining 20 preparations the contents of dialysable ^{131}I varied from 6.3 to 56.1 per cent of total activity as determined 1—3 weeks after the day of manufacture. In 11 batches less than 20 per cent of ^{131}I was dialysable and in 3 of these it was less than 10 per cent. Repeated determination showed a decrease of non-dialysable ^{131}I-fraction with increasing age in the older preparations, whereas the later ones seemed to be more stable (Fig. 1).

The stability of ^{131}I-PVP in feces was examined by incubation of homogenized stools with a known amount of ^{131}I-PVP (room temperature). The dialysable ^{131}I-fraction was unchanged after 3, 24 and 72 hours of incubation.

Fecal ^{131}I after oral supply of ^{131}I-PVP.

After oral intake of labelled PVP, 10 patients excreted from 36 to 87 per cent of the ^{131}I in their stools. 7 excreted more than half of the ingested activity. Each person had a different preparation. The subject who had the preparation with the lowest fraction of non-dialysable ^{131}I excreted less ^{131}I in the stools than the rest, but otherwise no correlation was found between fecal activity and non-dialysable activity *in vitro* (Fig. 2).

The concomitant urinary ^{131}I-excretion ranged from 3 to 13.5 per cent of the dose supplied in 9 subjects. The tenth — with the low fecal ^{131}I-output — excreted 46 per cent in her urine.

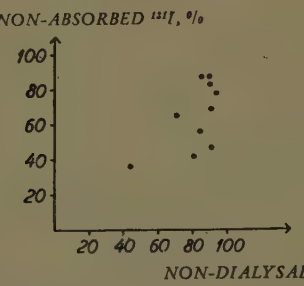


Fig. 2. The relation between non-dialysable (*in vitro*) and non-absorbed fraction of ^{131}I in orally supplied ^{131}I-PVP.

The intravenous ^{131}I-PVP test in control subjects.

The results of the PVP test in 22 controls are listed in Table I. Eighteen different batches were employed. The *mean cumulative fecal ^{131}I-output in 5 days* following injection was 0.52 per cent of the injected dose and the range (mean ± 2 × SD) (0 —1.01 per cent. The highest excretion actually found was 1.00 per cent. An attempt to correlate the normal ^{131}I-excretion with the amount of non-dialysable ^{131}I-PVP preparation failed to demonstrate any such relationship. Thus the mean excretion was 0.56 per cent in 14 subjects having batches with less than 20 per cent dialysable ^{131}I and 0.46 per cent in 8 subjects who had batches with from 20 to 52 per cent dialysable ^{131}I. The difference between these mean values was not significant ($40 > p > 30$).

Table I. ^{131}I-PVP-*test in 22 control patients.*

	Diagnosis	Serum Albumin g/100 ml	Cumulative fecal ^{131}I–output in 5 days % of inj.dose	^{131}I–PVP preparation	Per cent dialysable in vitro (24 hour dialysis
1. O.P. ♀	Disc degeneration		0.34	052158	10.4
2. G.C. ♂	Sciatica		0.75	071058	29.5
3. H.P. ♂	Congenital heart failure		0.37	—	—
4. C.C. ♂	Obesity	4.18	0.91	080758	10.0
5. K.O. ♂	Postgastrect. syndrome	4.56	0.41	090458	13.1
6. P.J.N. ♂	Epilepsia?	4.40	0.34	—	—
7. E.A. ♂	Parox. auric. flutter	4.61	0.88	—	—
8. L.S. ♀	Sciatica	4.34	0.44	102358	9.5
9. B.P. ♀	Cerebral concuss.	4.61	0.64	111358	19.3
10. O.H. ♂	Essent. hypertens.		0.94	120458	34.1
11. P.M.H.♂	Duod. ulcer?	4.72	1.00	010859	14.9
12. P.S. ♂	Duod. ulcer	4.46	0.66	050759	9.3
13. W.K.N.♂	Gastritis	4.01	0.60	—	—
14. K.H. ♂	Gastritis?	4.26	0.20	030359	15.8
15. C.K. ♂	Polycytemia	5.00	0.44	070959	6.3
16. S.V. ♂	Cerebral concuss.	4.37	0.57	080659	13.3
17. Å.M. ♂	Dyspepsia	4.55	0.28	110559	52.3
18. S.Å.C.♂	Duod. ulcer	4.59	0.35	120359	27.2
19. K.G. ♂	Disc degeneration	4.24	0.28	020460	43.5
20. P.P. ♂	Dyspepsia	5.20	0.40	080460	17.2
21. S.A. ♂	Coronary infarct.	3.94	0.54	090860	47.0
22. L.A. ♂	Pelvic fracture	3.80	0.17	100660	26.0
	Mean		0.52		
	Normal range (Mean ± 2 SD)		0.03–1.01		

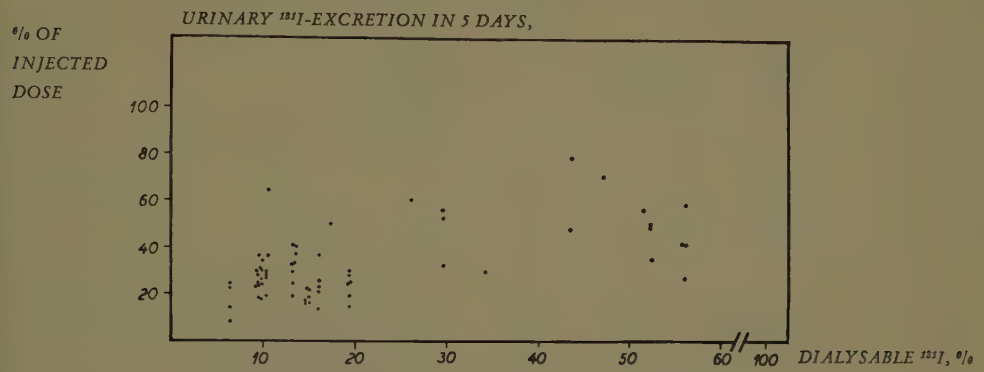


Fig. 3. Cumulative urinary ^{131}I-excretion (5 days) following intravenous injection of ^{131}I-PVP related to *in vitro* dialysable ^{131}I-fraction.

Urinary ^{131}I-excretion seemed to be lower ter ^{131}I-PVP with a small fraction of dia- sable ^{131}I (Fig. 3), but no definite relation s present. Generally it ranged from 20 to per cent of the injected dose.

The fraction of non-dialysable ^{131}I in urine s high during the first 24 hours following e injection and parallelled that of the PVP- eparation applied.

The apparent *initial distribution volume* s found to exceed the plasma volume by to 120 per cent. A rough correlation was esent between distribution volume and the ction of dialysable ^{131}I *in vitro* as demon- ated in Fig. 4.

In view of the high uptake of PVP by the iculo-endothelial system (Ravin, Selig- n & Fine, 1952) it was interesting that an raordinarily large distribution volume 30 per cent of plasma volume) was found a patient with generalized lymphosar- na.

An analysis of *plasma activity disappear- e* revealed that it followed an approximate ponential decline the first two or three rs. The slope constant was 0.03—0.10 hour^{-1}. There was no relation between dis- appearance rate and the relative content of dialysable activity. Generally the slope con- stant decreased after 2—3 days, but different patterns of activity curves were seen (Fig. 5) suggesting that the process of elimination was not a simple one.

The relation between non-dialysable ^{131}I in plasma and urine was studied in several

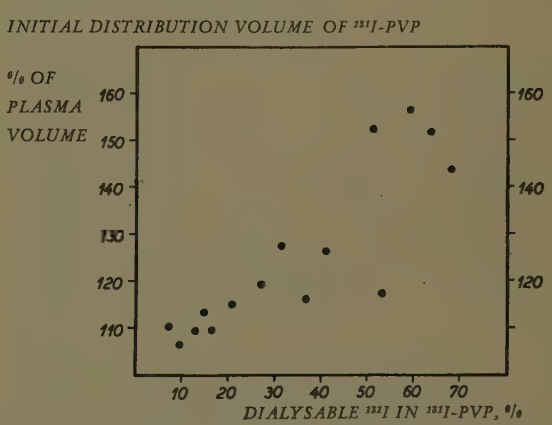


Fig. 4. Relation between initial distribution volume and contents of dialysable ^{131}I of 15 different PVP-praparations. Each dot represents one or the aver- age of up to 6 simultaneous "volume" determina- tions with ^{131}I-PVP and Evans Blue.

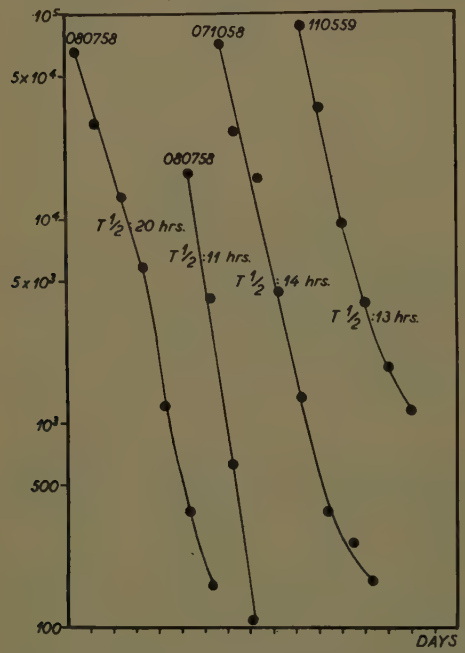


Fig. 5. Plasma activity following intravenous injection of ^{131}I-PVP. The first dot on each curve represents the activity 15 minutes after the injection.

subjects receiving three different batches of ^{131}I-PVP. In Fig. 6 the typical design is given: In plasma the relative content of non-dialysable ^{131}I remained constant or even increased somewhat, in urine the total amount of non-dialysable ^{131}I roughly parallelled the mean activity of plasma within the same 24 hours. The renal clearance (average of day 2-3-4) of non-dialysable ^{131}I was about 33 per cent of the plasma volume per 24 hours in the 2 control subjects shown in Fig. 6 (preparation 080460: 21-32-26, and preparation 100660: 47-35-24 per cent per day). This is 3 times higher than the normal metabolic clearance of ^{131}I-albumin (which is about 10 per cent of the circulating albumin per day).

The ^{131}I-PVP test in hypoproteinemic subjects.

Idiopathic hypoproteinemia.

Table II includes 7 patients with gast diseases (Nos. 1—7) and 5 with unkno intestinal diseases (Nos. 8—12). Serum alb min was low in all of them and most of the had marked edema. In 9 of them a hyp catabolic hypoalbuminemia was present (

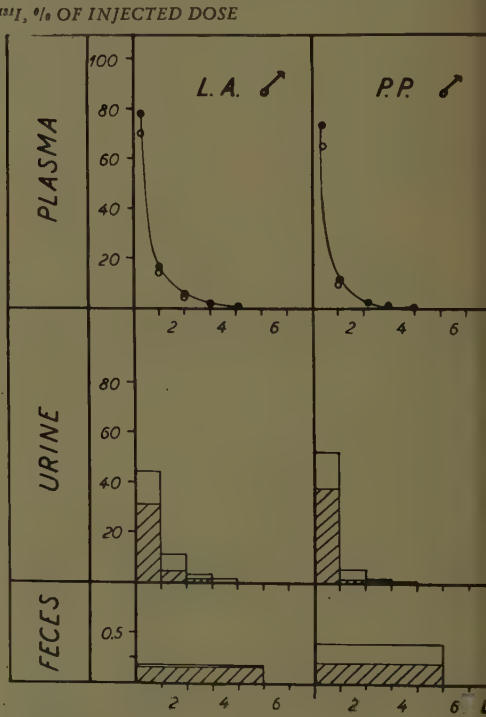


Fig. 6. Intravascular contents and excreted amou of ^{131}I following intravenous injection of ^{131}I-P *Left:* ^{131}I-PVP 100660 with 74 per cent non- lysable ^{131}I. *Right:* ^{131}I-PVP 080460 with 83 cent non-dialysable ^{131}I.

●: non-dialysable ^{131}I in plasma as per cent of jected non-dialysable ^{131}I.

o: total plasma ^{131}I, per cent of total amount ^{131}I injected.

////: non-dialysable ^{131}I-fraction.

Table II. *Serum albumin, ^{131}I-albumin metabolism and ^{131}I PVP-test in 12 patients with «idiopathic hypoalbuminemia».*

	Age	Diagnosis	Serum Albumin g/100 ml	Albumin turnover		Fecal ^{131}I in 5 days % of inj.dose
				Relative % of C.A.*/day	Absolute g/kg/day	
1. K.F. ♂	65	Hypertroph. gastritis	3.16	11.0	0.17	1.75
2. E.F.H.♀	65	Hypertroph. gastritis	2.99	12.8	0.16	2.95
3. H.M. ♂	62	Giant hyp. gastritis	2.45	13.4	0.32**	3.38
3a H.M.		After part. gastrect.	3.78	8.0	0.16	1.03
4. E.N. ♂	33	Hypertroph. gastritis	2.72	17.9	0.27	1.46
5. W.D. ♂	72	Gastric papilloma	1.02	19.8	0.09	3.31
5a W.D.		After part. gastrect.	4.68			0.16
6. S.T. ♀	63	Ulc. gastrit. + postgastrect.	2.29	20.5	0.24	4.47
7. S.Å.L.♂	34	Giant hyp. gastritis	1.11	29.0	0.39	3.41
8. B.P. ♀	49	Unknown intest. disease	1.88	26.2	0.21	5.93
9. R.L. ♂	38	Unknown intest. disease with granulomas	2.01	29.3	0.43	7.95
10. K.K. ♀	28	Unknown intest. disease	3.01	30.4	0.37	3.14
11. E.S. ♀	30	Unknown intest. disease	2.45	34.6	0.34	4.14
12. D.S.N. ♀	½	Unknown intest. disease	1.69	62.4	0.68	4.6/2 days
		Normal average and range (Mean ± 2 SD) (Jarnum & Schwartz, 1960)	4.41 3.77–5.05	10.0 5.6–14.4	0.206 0.13–0.28	0.52 0.03–1.01

* C.A. = circulating albumin.
** In H. M. ^{131}I-albumin study was done after intravenous infusion of albumin.

radation rate exceeding 14.4 per cent of circulating albumin per 24 hours (Jarnum & Schwartz, 1960)). An abnormal PVP test was found in every case of this series. In two patients the test became normal after partial gastrectomy (No. 3 with giant hypertrophic gastritis and No. 5 with a gastric papilloma) and serum albumin increased spontaneously to a normal level. Extensive intestinal disease of undetermined nature was found at laparotomy in 4 of the 5 patients with protein-losing enteropathy.

Cancer patients.

An abnormal test was found in 8 of 9 cases of gastric carcinoma (Table III). Two non-anemic patients had carcinoma of the colon. The test was normal in the nonmoproteinemic one, and abnormal in the other who had a marked hypoalbuminemia. In 2 hypoalbuminemic patients with malignant disease outside the gastrointestinal tract (Nos. 12 and 13) the test was normal.

Chronic diarrhea.

A marked increase of fecal ^{131}I was found in one patient with regional enteritis and in one with active ulcerative colitis (Nos. 2 and 3 respectively in Table IV). In 2 patients with moderate steatorrhea (Nos. 5 and 6)

Table III. ^{131}I-PVP-*test in 13 cancer patients.*

	Age	Diagnosis	Serum Albumin g/100 ml	Fecal ^{131}I, % of inj.dose	Metastatic lesions of live
1. N.L. ♀	85	Gastric carcinoma + pernic. anemia	2.80	0.87	?
2. E.S. ♂	54	Gastric carcinoma	2.59	1.13	+
3. R.C. ♂	69	Gastric carcinoma	2.19	1.17	—
4. Z.J. ♂	77	Gastric carcinoma	2.57	1.32	+
5. L.N. ♂	67	Gastric carcinoma	2.91	1.70	—
6. K.H. ♀	53	Gastric carcinoma	4.01	1.78	—
7. P.L. ♂	54	Gastric carcinoma	2.17	1.79	+
8. P.R. ♂	74	Gastric carcinoma	2.99	4.25	—
9. O.C. ♂	72	Gastric carcinoma	2.01	7.32	—
10. K.P. ♀	65	Carc. coli sigmoid.	4.05	0.60	—
11. C.L. ♂	75	Carc. coli ascend. + polyposis coli	2.04	2.16	—
12. E.C. ♀	62	Carc. ovarii with ascites	1.35	0.30	?
13. O.G. ♂	24	Generalized lymphosarcoma	2.21	0.15	+
		Normal range	3.8–5.1	0.03–1.01	

Cases Nos. 1—11: carcinoma in the gastrointestinal tract.
Cases Nos. 12—13: cancer outside the digestive tract.

Table IV. ^{131}I-PVP-*test in 8 patients with chronic diarrhea of various etiology.*

	Age	Diagnosis	Serum Albumin g/100 ml	Albumin turnover		Fecal ^{131}I in 5 days % of inj.dose	Comment
				Relative % of C.A./day	Absolute g/kg/day		
1. T.K. ♂	19	Regional enteritis	1.90	13.4	0.15	1.01*	
2. S.M. ♀	35	Regional enteritis	2.70	8.3	0.13	2.86	
3. K.S. ♀	39	Ulcerat. colitis	2.88			3.56	
4. M.F. ♀	53	Ulcerat. colitis (quiescent phase)	3.44			0.85	
5. J.B.P. ♂	65	Steatorrhea	2.59	11.9	0.23	0.34	Remission on gluten-free regim
6. A.L. ♀	44	Malabsorpt. syndrome (non-tropical sprue ?)	3.61			0.56	
7. J.B. ♂	62	Chron. pancreatitis with steatorrhea	2.74	9.6	0.15	1.25	
8. K.U. ♀	66	Chron. diarrhea (in remiss.)+idiopath. hypoproteinemia	2.19			1.35	
		Normal range	3.8–5.1	5.6–14.4	0.13–0.28	0.03–1.01	

*Poor ^{131}I-PVP preparation (090359). Almost all ^{131}I turned out to be dialysable.

e test was normal, in one (No. 7) with vere steatorrhea due to chronic pancreatitis was slightly abnormal.

The last patient (No. 8) had suffered from arrhea for 2 months following an arthrodesis of e left knee-joint. She had a marked hypoalbumin-ia (about 2 g per 100 ml) and a slightly elevated mma globulin (about 1.5 g per 100 ml), but herwise no signs of hepatic disease. Galactose d bromsulfalein tests were normal. Her diarrhea d stopped. An intestinal series showed a patchy sign. The PVP test was slightly abnormal. rther studies could not be undertaken in this tient who might have had a transitory protein-sing enteropathy although the mechanism of her poalbuminemia was probably complex.

ostgastrectomy syndrome.

An abnormal test was found in 4 patients ith postgastrectomy syndrome associated ith steatorrhea (Table V). Fecal ^{131}I-xcretion was highest in the patient (No. 7) ith the lowest serum albumin. Two patients with still lower serum albumin displayed a normal test, but both had fatty liver (biopsy finding).

Hepatic and renal diseases.

This group included 7 patients who all had a normal fecal ^{131}I-output (Table VI). In 2 patients with nephrotic syndrome (Nos. 4 and 6) the "endogenous" degradation rate of ^{131}I-albumin (calculated from non-protein-bound urinary activity) was abnormally high. This would suggest an "idiopathic" component in their hypoalbuminemia beside the urinary protein loss. In one of them (No. 4), who suffered from extensive primary amyloidosis (including the gastrointestinal tract), a considerable amount of the label was lost in vomitus. He might have passed part of it in the stools had vomiting not occurred.

Table V. ^{131}I-PVP-*test in 7 patients with postgastrectomy syndrome and hypoalbuminemia.*

	Age	Diagnosis	Serum Albumin g/100 ml	Albumin turnover Relative % of C.A./day	Absolute g/kg/day	Fecal ^{131}I in 5 days % of inj.dose	Hepatic Affection
S.W.N. ♂	42	Postgastrectomy + hemofuscinosis duodeni	3.47			0.29	—
G.F. ♀	43	Postgastrect. + anemia	2.72	6.1	0.12	0.36	+(fatty infiltr. + slight fibrosis*)
H.C.A. ♂	48	Postgastrect. + steatorrhea	2.51	9.0	0.07	0.48	+ (fatty liver*)
N.P. ♂	40	Postgastrect. + hypertroph. gastritis + steatorrhea	3.83			1.34	—
T.N. ♂	48	Postgastrect. + steatorrhea	3.70			1.53	—
R.V. ♀	43	Postgastrect. + steatorrhea	3.57	10.6	0.25	1.55	—
H.E.L. ♂	57	Postgastrect. + steatorrhea	2.76	9.0	0.17	2.55	(+)
		Normal range	3.8–5.1	5.6–14.4	0.13–0.28	0.03–1.01	

Liver biopsy.

Table VI. ^{131}I-PVP *test and albumin metabolism in 3 patients with hepatic diseases and 4 with nephrotic syndromes.*

	Age	Diagnosis	Serum Albumin g/100 ml	Albumin turnover		Fecal ^{131}I in 5 days % of inj.dose	Comments
				Relative % of C.A./day	Absolute g/kg/day		
1. B.E. ♀	74	Chronic hepatitis?	2.53			0.02	
2. T.V. ♂	56	Cirrhosis + postgastrectomy	1.35			0.23	
3. M.P. ♀	65	Chronic hepatitis in remission	3.11	18.5	0.19	0.34	*Liver biopsy :* fatty with slig fibrosis & mar ked regene-
4. S.S. ♂	42	Primary amyloidosis +nephrotic syndrome +postgastrectomy	1.72	23.7(19.1)*	0.21	0.11	ration. *In vomitus :* 4.05% of inj.^{1}
5. E.J. ♀	21	Nephrosis	1.50	20.9(9.9)*	0.12	0.28	
6. J.J. ♂	62	Nephrosis	1.09	45.5(23.8)*	0.21	0.29	
7. P.L. ♂	60	Nephrosis + postgastrectomy	1.25	21.4(11.6)*	0.12	0.82	
		Normal range	3.8–5.1	5.6–14.4	0.13–0.28	0.03–1.01	

*Parenthetically the degradation rate calculated from non-proteinbound urinary ^{131}I.

Relation between serum albumin and fecal ^{131}I in hypoalbuminemia.

A theoretical relationship between fecal ^{131}I and serum albumin could be calculated assuming: a) a linear relationship between the 3 parameters: fecal ^{131}I-output in excess of the normal upper limit (1 per cent of the injected dose), the abnormal gastrointestinal protein loss, and albumin degradation rate in excess of normal mean (10 per cent of circulating albumin per day), b) a constant albumin production at all serum albumin levels.

Curve B (Fig. 7) gives this relation in the 10 patients with idiopathic hypoalbuminemia. Taking the mean figures of serum albumin, albumin degradation rate and PVP test from this group it was found that an increase of fecal ^{131}I by 1 per cent of the injected dose would correspond to an increase in albumin degradation rate of about 5.3 per cent circulating albumin per day. This figure w applied in the construction of curve A fr the mean figures of serum albumin and alb min degradation rate in the normal contr The latter curve thus illustrates serum bumin concentration at a certain prot leakage if albumin *production* remained changed. Now, two conclusions may drawn from Fig. 7:

1) ^{131}I-excretion in the stools of patie with idiopathic hypoproteinemia was versely related to serum albumin.

2) The serum albumin at a certain ^{1} excretion level was considerably hig (about 40 per cent) in idiopathic hypoal minemia than anticipated from the "norm curve A, or — in other words — an incre of albumin *production* takes place in idio thic hypoproteinemia.

ide effects.

In two patients a typical *"colloid reaction"* ccurred. This reaction is characterized by nest and back pain together with a marked ushing of the skin (especially of the face) nd develops within a minute of the injection. In one case the reaction was very dra- natic: The patient lost consciousness (and ulse and respiration as well), probably artly because he was weak and anemic Jarnum & Schwartz, 1960), but he quickly ecovered. Except these two reactions no un- ward effects have been observed.

The colloid reaction must be ascribed to ne colloid nature of the PVP-preparation. dentical reactions have been described after ntravenous administration of other colloidal ubstances (inulin, iron-dextran, fat emul- ions (Preston & Henegar, 1959)).

DISCUSSION

PVP has been extensively used as a plasma expander since its introduction in Germany about 20 years ago (Hecht & Weese, 1943). A detailed report on its kinetics and distribution has been published by Ravin, Seligman & Fine (1952). Working with ^{131}I- and ^{14}C-labelled PVP, they demonstrated that the amount metabolized after intravenous injection was quite insignificant. Their data on urinary excretion (40—80 per cent of the injected dose, inversely related to molecular size) agree well with those presented here, as does plasma disappearance rate which was about 80 per cent from 5 minutes till 12 hours after injection for the fractions with molecular size from 30,000 to 40,000. Of special interest was the small fecal excretion, below 0.6 per cent of the injected dose in the first 5 days, in both man and dog. Furthermore, the fecal excretion could be shown to stem exclusively from bile in dogs.

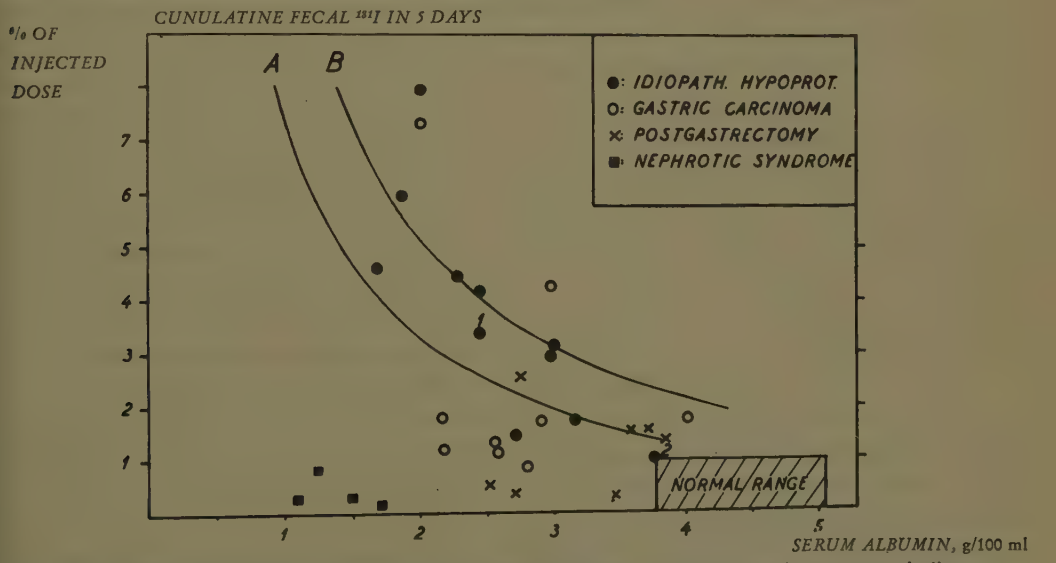


Fig. 7. Relation between fecal ^{131}I-PVP and serum albumin concentration. The curves indicate the theoretical relationship (see text) at normal albumin production (A) and in idiopathic hypoproteinemia (B). 1—2: Giant hyperthropic gastritis before and after partial gastrectomy.

The ^{131}I-PVP made by Ravin *et al.* was unsuitable as test substance in gastrointestinal protein loss, because it turned out to be unstable (Gordon 1958). With Gordon's preparation a more stable ^{131}I-PVP can be obtained. In both preparations the ^{131}iodine is added to the terminal double bond of PVP, in the older one directly to the carbon atom and in Gordon's as a p-^{131}I-phenyl group.

In the present report a slight liberation of ^{131}I on standing was demonstrated only in the older preparations (Fig. 1), and no decomposition took place when ^{131}I-PVP was added to homogenized feces. The stability could thus be confirmed.

As no fractionation of the ^{131}I according to molecular size was undertaken, the simple procedure of dialysis through a cellophane membrane was employed to evaluate the relative contents of large molecules. A reasonably good agreement was in this way obtained between dialysability *in vitro* and *"in vivo"*, the latter denoting the fraction which disappeared from the blood stream in the first 15 minutes following the intravenous injection (Fig. 4).

It was found that the renal clearance of large molecules amounted to about ⅓ of the plasma volume per 24 hours. This is virtually a small figure compared with glomerular filtration rate (about 50 times the plasma volume per day).

Whether a renal clearance of ⅓ of the plasma volume per 24 hours is comparable with the glomerular filtration rate of serum albumin can not be assessed with present knowledge, as no quantitative data on glomerular leakage of plasma proteins are at hand (Rather, 1952).

A direct comparison between gastrointestinal clearance of "cellophane-retained" PV molecules and albumin degradation in idi pathic hypoproteinemia was not possib from the data in the present report. O might expect from the heterogeneity of t preparations that no simple correlati existed. Young *et al.* (1959) made such correlation in one patient and found a re sonably good agreement. However, until pr parations of a more uniform molecular si are available, it seems important that in t present series all preparations with a no dialysable fraction of about 50 to 94 per ce ^{131}I were very useful in the establishment an abnormal gastrointestinal protein loss.

The clinical material investigated in th report (details are reported elsewhe (Schwartz & Jarnum)) showed that in a 12 cases of *idiopathic hypoalbuminemia* a abnormal PVP test could be demonstrate The fact that 2 patients (Table II, Nos. and 5) became normoproteinemic and sho ed a normal test following surgical resecti of the presumed protein-leaking lesion, ga further evidence of the validity of the test.

The slightly abnormal test met with *gastric carcinoma* suggested a double mec anism of the hypoalbuminemia in this co dition. This was not surprising consideri that hypoalbuminemia associated with a no mal test may follow malignant disease ou side the gastrointestinal tract even befo weight loss and malnutrition set in (Tab III). The demonstration of an abnormal te in a hypoproteinemic case of carcinoma the colon is an indication that a plasma pr tein leakage may be associated with mali nant disease everywhere in the gastrointe inal tract. Kippen, Palmer & Kirsner (194 described a case of lymphoblastoma in t

small intestine with severe hypercatabolic hypoalbuminemia (demonstrated by the rapid disappearance of infused plasma). They did not discuss the low serum protein.

The occurrence of protein-leakage in *regional enteritis* and *ulcerative colitis* is now well established (Steinfeld *et al.*, 1960, Vesin *et al.*, 1960).

An abnormal test may also be found in *steatorrhea*, which is often associated with hypoproteinemia. Thus Parkins (1960) found an abnormal test in 4 of 11 cases of sprue syndrome (all with atrophic mucosa in intestinal biopsies), but strangely enough there was no tendency for higher fecal ^{131}I-excretion in patients with low serum albumin. In the present report an abnormal test was found in one case of chronic pancreatitis and in 4 postgastrectomy syndromes associated with steatorrhea. Vesin *et al.* (1960) reported a case of chronic jejuno-ileitis with steatorrhea and marked gastrointestinal protein loss, and a similar case has been described by Schwartz & Jarnum (1959) (here listed in Table II as No. 11). They found this combination in another patient too who suffered from intestinal granulomatosis (Table II, No. 9). An identical mechanism was highly probable in a case of massive jejunal diverticulosis reported by Watkinson, Feather, Marson & Dossett (1959). The patient, a 64-year-old man, had severe steatorrhea with marked hypoproteinemia (4.3 g per 100 ml) and Addisonian-like anemia. The latter could be quite succesfully treated by B_{12} and folic acid, but resection of the diseased jejunum resulted in complete remission.

Thus gastrointestinal protein loss can be related to not less than 5 (probably 6) different types of steatorrhea. On the other hand, a review of the world literature on idiopathic hypoproteinemia (133 patients) revealed that steatorrhea had been a concominant finding in 30 patients. No steatorrhea was present in 21 patients. In the remaining 82 the authors did not mention the fat contents of the stools.

The interrelationship between abnormal protein loss and steatorrhea is remarkable. Both findings may reflect an abnormally high transfer of protein- and fat-rich fluid (the source of the chylus) from the interstitial space of the villi to the lumen of the gut, but one is not in a position to say whether this is due to an abnormal epithelial leakage, to obstruction of the efferent pathways (*i. e.* intestinal lymphatics), or merely to an increased formation of interstitial fluid. The high incidence of chylous effusions (especially chylous ascites) and dilated lymphatics in the small intestine (Gordon *et al.*, 1959) in idiopathic hypoproteinemia are indicators of a lesion or an abnormal function of the intestinal lymphatic apparatus.

The *relation between serum albumin and fecal ^{131}I-excretion* following intravenous ^{131}I-PVP in idiopathic hypoproteinemia was roughly the same as that predicted and found by Gordon (Gordon *et al.*, 1959) in his 11 cases. The normal ^{131}I-excretion lay below 1.01 per cent of the injected dose. It is not possible to decide whether this originated from the normal "leakage" of plasma proteins (Holman, Nickel & Sleisenger 1959, Armstrong, Margen & Tarver 1960, Wetterfors, Gullberg, Liljedahl, Plantin, Birke & Olhagen 1960). It may be so as fecal ^{131}I is appreciably higher than after intravenous ^{131}I-albumin which is digested in

the gut with the result that most of the label is reabsorbed (leaving less than 0.4 per cent in the stools (Jarnum, 1961)). On the other hand it may have been excreted in the bile like the ^{14}C-labelled PVP applied by Ravin *et al.* (1952).

SUMMARY

Experience with Gordon's ^{131}I-PVP as a test substance in gastrointestinal protein loss is reported.

In vitro and after incubation with homogenized stools, the fraction of non-dialysable ^{131}I remained essentially constant.

79 patients were studied.

10 had *oral ^{131}I-PVP* and lost from 36 to 87 per cent of the label in the stools.

22 *control subjects* had *intravenous ^{131}I-PVP*. The cumulative mean ^{131}I-excretion in feces in a 5-day period was 0.52 per cent of the injected dose, range (mean $\pm$ 2 $\times$ SD): 0.03—1.01 per cent for 20 different batches containing a dialysable ^{131}I-fraction of 6—52 per cent (determined by dialysis through a cellophane membrane). The initial distribution volume of ^{131}I-PVP (15 minutes after injection) was directly related to the dialysable fraction of the injected PVP, and urinary ^{131}I-excretion was less when the fraction of non-dialysable ^{131}I was high.

A PVP test was performed in 47 patients of whom the great majority was hypoproteinemic. It was abnormal in all 12 cases of *idiopathic hypoproteinemia,* in 8 with *gastric carcinoma* and 1 with *carcinoma of the colon,* in 1 with *regional enteritis* and 1 with *ulcerative colitis,* and in 7 patients with *steatorrhea* of various origin.

The remarkably high coincidence of abnormal gastrointestinal protein loss, steatorrhea, dilated intestinal lymphatics and chylous effusions is discussed. It may refle an impaired or insufficient drainage of t interstitial fluid of the villi.

An inverse correlation was found betwe serum albumin concentration and fecal ^{131} output in idiopathic hypoproteinemia. It concluded that, at present, an abnormal PV test is the most reliable indicator of a gastr intestinal protein leakage and the only o to permit a direct — although relative estimation of the size of the leakage.

REFERENCES

Allen, T. H., Ochoa, M. Jr., Roth, R. F. & Greg sen, M. I.: Spectral absorption of T-1824 plasma of various species and recovery of t dye by extraction. Am. J. Physiol. *175,* 2 1953.

Armstrong, F. B., Margen, S. & Tarver, H Plasma protein VII. Site of degradation serum albumin. Proc. Soc. Exper. Biol. Med. *103,* 592, 1960.

Gordon, R. S. Jr.: The preparation of radioact polyvinylpyrrolidone for medical use. J. Po mer Sc. *31,* 191, 1958.

— Exudative enteropathy. Abnormal permeabil of the gastrointestinal tract demonstrable w labelled polyvinylpyrrolidone. Lancet *1,* 3 1959.

— Bartter, F. C. & Waldmann, Th.: Idiopat hypoalbuminemias: Clinical staff conference the National Institute of Health. Ann. Med. *51,* 553, 1959.

Hecht, G. & Weese, H.: Periston, ein neuer Bl flüssigkeitsersatz. München. med. Wchnsc *90,* 11, 1943.

Holman, H., Nickel, W. F. Jr. & Sleisen M. H.: Hypoproteinemia antedating intest lesions, and possibly due to excessive se protein loss into the intestine. Am. J. Med. 963, 1959.

Hsia, D. Y. Y., White, H. & Hugh-Jones, Idiopathic hypoproteinemia. Demonstration gastrointestinal protein loss through use I^{131} labelled P.V.P. Clin. Research *7,* 392, 1

rnum, S.: A modified extraction method for determination of Evans Blue. Scandinav. J. Clin. & Lab. Investigation *11*, 332, 1959.

- Fecal ^{131}I-output after intravenous injection of ^{131}I-labelled human serum albumin in normo- and hypoproteinemic subjects. To be published.

- & Schwartz, M.: Hypoalbuminemia in gastric carcinoma. Gastroenterology *38*, 769, 1960.

- & Schwartz, M.: Studies of albumin catabolism by means of ^{131}I-labelled albumin. Methods of calculating the albumin turnover and some fields of clinical application. Nord. med. *63*, 708, 1960.

ppen, D. L., Palmer, W. L. & Kirsner, J. B.: Lymphoblastoma of the small intestine. Gastroenterology *12*, 302, 1949.

urell, C.-B., Laurell, S. & Skoog, N.: Buffer composition in paper electrophoresis: Considerations on its influence with special reference to the interaction between small ions and proteins. Clin. Chem. *2*, 99, 1956.

artini, G. A., Strohmeyer, G. & Bünger, P.: Exudative Enteropathie ("essentielle Hypoproteinämie") mit rezidivierender Gelbsucht und Verkalkungen in Bauchraum. Deutsche Med. Wchenschr. *85*, 586, 1960.

atthews, C. M. E.: The theory of tracer experiments with ^{131}I-labelled plasma proteins. Phys. in Med. Biol. *2*, 36, 1957.

arkins, R. A.: Protein-losing enteropathy in the sprue syndrome. Lancet *2*, 1366, 1960.

earson, J. D., Veall, N. & Vetter, H.: A practical method for plasma albumin turnover studies. Radioakt. Isot. in Klin. Forsch. *3*, 290, 1958.

Protein-losing Gastroenteropathy". Leading article. Lancet *1*, 351, 1959.

reston, F. W. & Henegar, G. C.: Use of intravenous fat emulsions in surgical patients. Surg. Clin. North America *39*, 145, 1959.

ather, L. J.: Filtration, resorption, and excretion of protein by the kidney. Medicine *31*, 357, 1952.

avin, H. A., Seligman, A. M. & Fine, J.: Polyvinyl pyrrolidone as a plasma expander. Studies on its excretion, distribution and metabolism. New England J. Med. *247*, 921, 1952.

oyer, P.: Hypoprotéinémie chronique idiopathique portant sur les fractions albumine et gammaglobulines, liée à une intolérance au gluten. Ann. Pédiat. (Paris) *36*, 231, 1960.

Schwartz, M. & Jarnum, S.: Gastrointestinal protein loss in idiopathic (hypercatabolic) hypoproteinemia. Lancet *1*, 327, 1959.

— & Jarnum, S.: Protein-losing gastroenteropathy of various etiology, diagnosed by means of ^{131}I-albumin. Dan. Med. Bull. *8*, 1, 1961.

Steinfeld, J. L., Davidson, J. D., Gordon, R. S. Jr. & Greene, F. E.: The mechanism of hypoproteinemia in patients with regional enteritis and ulcerative colitis. Am. J. Med. *29*, 405, 1960.

Watkinson, G., Feather, D. B., Marson, F. G. W., & Dossett, J. A.: Massive jejunal diverticulosis with steatorrhea and megaloblastic anemia improved by excision of diverticula. Brit. Med. J. *2*, 58, 1959.

Veall, N., Pearson, J. D. & Hanley, T.: The preparation of ^{132}I- and ^{131}I-labelled human serum albumin for clinical tracer studies. Brit. J. Radiol. *28*, 633, 1955.

Vesin, P., Renault, H. & Cattan, R.: L'hypoprotéinémie idiopathique. Étude de son mécanisme par le test au polyvinylpyrrolidone-I^{131}. Bull. et mêm. Soc. mêd. d. hôp. de Paris *75*, 319, 1959.

— Troupel, S., Acar, J., Bismuth, V., Renault, H. & Cattan, R.: Iléocolite de Crohn. Étude du metabolisme intestinal par la trioléine-I^{131} et le PVP-I^{131} Bull. et mêm. Soc. mêd. d. hôp. de Paris *76*, 410, 1960.

— Troupel, S., Acar, J., Renault, H., Desbuquois, G. & Cattan, R.: Entéropathie avec perte de protéines et stéatorrhée. Étude par le PVP-I^{131} et la trioléine-I^{131}. Action du régime sans gluten. Bull. et mêm. Soc. mêd. d. hôp. de Paris *76*, 261, 1960.

Wetterfors, J., Gullberg, R., Liljedahl, S.-O., Platin, L.-O., Birke, G. & Olhagen, B.: Role of the stomach and small intestine in albumin breakdown. Acta med. Scandinav. *168*, 347, 1960.

Young, W. F., Fowler, J. F., Barnett, L. & Miles, J. M.: Gastrointestinal protein loss in severe malabsorption complicated by idiopathic (hypercatabolic) hypoproteinemia. Lancet *2*, 85, 1959.

Scandinav. J. Clin. & Lab. Investigation 462—475, 13, 1961.

FECAL ^{131}I-OUTPUT AFTER INTRAVENOUS INJECTION O ^{131}I-LABELLED HUMAN SERUM ALBUMIN IN NORMO- AND HYPOPROTEINEMIC SUBJECTS[1]

By S. JARNUM

(with the technical assistance of Bodil Møller-Petersen and Lis Hansen)

From the Department of Clinical Biochemistry and Medical Department B, Bispebjerg Hospital Copenhagen, Denmark

(Received for publication February, 1961)

The introduction of ^{131}I-labelled polyvinylpyrrolidone (^{131}I-PVP) (Gordon, 1959) has made it possible to establish the presence of an abnormal gastrointestinal leakage of plasma proteins. After intravenous injection of the high molecular polymere, an abnormally high amount will escape from the blood to the gastrointestinal tract and can be recovered in the stools as it is left unchanged by the digestive enzymes.

Similarly, some of the intravenously supplied ^{131}I-albumin will leak out, but being a protein it is split by enzymatic breakdown, and most of the label is reabsorbed and leaves the body *via* the kidneys. Only a fraction of ^{131}I remains in the gut and the amount eventually passed on in the stools will be much less than the amount leaked out. Actually this was the rationale for introducing an inert substance like PVP.

However, no report has been published on the fecal ^{131}I-excretion following intravenous ^{131}I-albumin injection in normoproteinemic subjects and patients suffering from hypoproteinemia of various origin. Below such investigations are reported together wit comparison of ^{131}I-albumin and ^{131}I-PV diagnostic tools in protein-losing gas enteropathy.

PATIENTS

Control patients.

15 patients with normal serum albumin con tration served as controls (Table II). None fever, all had a normal hemoglobin concentra and erythrocyte sedimentation rate. Most of t suffered from duodenal ulcer, but they were quiescent phase without vomiting or pains. All in a good nutritional state. One (No. 9) had b in his feces, another (No. 3) trace of blood, rest of them had no gastrointestinal bleeding 3 patients (1, 13 and 14) a mild diarrhea occu during the study.

Hypoproteinemic patients with gastrointestinal disorders.

28 patients with hypoproteinemia and va gastrointestinal diseases were investigated. were subdivided according to their ^{131}I-albumin gradation rate (for further discussion of this p see later). The normal degradation rate amoun 10 per cent of the intravascular albumin pool day (absolute value: about 0.2 g per kg per with a range of 5.6—14.4 per cent per day (Jar & Schwartz, 1960) based on the results of albumin studies in 20 hospitalized patients o ages suffering from a variety of diseases no fecting the serum-protein concentration.

Group A: 12 patients with hypercatabolic h albuminemia, *i. e.* a degradation rate exceeding

[1] This work was supported by grants from Statens Videnskabsfond, Kong Christian den X's Fond and Fonden af 21/12 1956 til Lægevidenskabens Fremme.

r cent of circulating albumin per day (Table III). of the patients had hypertrophic gastritis (Nos. 1 d 2), 1 gastric papilloma with malignant trans-rmation (No. 3), 1 ulcerative gastritis and post-strectomy syndrome (No. 4), 1 ulcerative colitis Jo. 5), 1 intestinal lymphogranulomatosis (No. 9), patients (Nos. 6-7-8-10) had unknown intestinal sease ("protein-losing enteropathy"). The last two ses (Nos. 11 and 12) were 2 otherwise normal cer patients studied in the immediate postopera-e period after partial gastrectomy.

Group B: included 16 cases with normocatabolic poalbuminemia (Tables IV and V). 4 patients d presumed hypertrophic gastritis (radiological idence). In one of these a giant hypertrophic stritis was found on partial gastrectomy. 4 suf-red from a postgastrectomy syndrome, 1 had stro-entero-anastomosis, 4 gastric carcinoma, 2 ginal enteritis and one the sequelae of massive testinal resection (all of the ileum except the stal 5 cm).

ypoproteinemic subjects without gastro-testinal disease.

Investigations were undertaken in 8 cases of poalbuminemia and diseases which did not pri-arily affect the gastrointestinal tract (Table VI). ne patient had myxœdema, 3 chronic infections d 4 nephrotic syndromes.

METHODS

^{131}I-albumin was prepared according to the ethod of Veall, Pearson and Hanley (1955). bout 99 per cent of the activity in the final pre-ration was precipitable by trichloracetic acid.

vitro experiments.

As a certain proteolytic activity is present in e stools, experiments were designed to evaluate e "fecal degradation rate" of admixed plasma oteins. Homogenized feces from 3 patients were cubated with ^{131}I-albumin and in an additional periment with Na^{131}I. After incubation (room mperature) for 1 and 24 hours, samples were thdrawn and dialyzed in cellophane bags against 9 per cent sodium chloride or running tapwater r 3 hours. TCA-precipitable activity was deter-ined at the same intervals. Protein- and iodine-rrier (plasma and 20 per cent potassium iodine respectively) was added to all homogenates previous to incubation.

The amount of non-dialysable ^{131}I spontaneously excreted in the feces was ordinarily not examined, partly because the activity was too small to obtain a reasonable accuracy.

Patient studies.

30—50 μC of *^{131}I-albumin* were injected intravenously. Blood was withdrawn after 15 minutes and at daily intervals for about 8 days. Plasma activity was measured in a well type scintillation counter. 5 days (120 hours) after the injection 1 g carmine was given *per os*. Stool collection began immediately after the injection and continued until the stools became red. They were mixed and homogenized in a blender after addition of water. The activity of 500 ml was measured in a scintillation counter designed for large volumes.

The same procedure was followed after intravenous injection of *^{131}I-PVP*.[1]

To prevent thyroid ^{131}I-uptake, 1.3 g potassium iodide was supplied daily for about a week commencing on the day of injection or 1-2 days before.

Nitrogen and fat analyses of feces were made in several of the hypoproteinemic subjects.

24-hour urinary specimens were counted for about 8 days.

Serum-protein was determined by Kjeldahl analysis, serum-albumin by paper electrophoresis (Laurell, Laurell & Skoog, 1956).

^{131}I-albumin turnover was calculated after the method of Pearson, Veall & Vetter. Where quantitative urine collection could not be ascertained, the method described by Matthews (1957) was applied (mathematical analysis of plasma activity curve).

RESULTS

In vitro experiments.

After a 24-hour incubation of ^{131}I-albumin with feces, an appreciable amount of protein-bound activity was still present (Table I), as 4 times as much ^{131}I was retained in a cello-

[1] ^{131}I-PVP was kindly supplied by Robert Gordon, Bethesda, U.S.A.

Table I. *Non-dialysable* ^{131}I *in feces after incubation in vitro with* ^{131}I*-albumin and* $Na^{131}I$.

	Diagnosis	Feces incubated with	Non-dialysable ^{131}I-fraction (%) after		Non-dialysable ^{131}I after addition of trypsin inhibitor 24 hours' incubation
			1 hour	24 hours	
1. R.L.	Steatorrhea, Protein-losing enteropathy	^{131}I-Albumin	92	85	91
2. M.	Int. Resection Steatorrhea	»	89	81	94
3. H.S.	Gastritis	»		90	
3a H.S.	»	$Na^{131}I$		22	

phane bag as after incubation with $Na^{131}I$.

This applied to both normal stools (No. 3) and stools from patients with steatorrhea (Nos. 1 and 2) and one with protein-losing enteropathy (No. 1).

10 per cent trichloracetic acid precipitated 86 and 39 per cent of the activity in Nos. 1 and 2 respectively after a 24-hour incubation. Preliminary addition of trypsin inhibitor[1] impaired the breakdown to some extent.

Fecal ^{131}I*-excretion in control patients.*

Fecal ^{131}I in 15 control patients amounted to an average of 0.14 per cent of the injected dose in 5 days, (Table II). Standard deviation (SD) was $\pm$ 0.08 per cent. As statistical inaccuracy of the net count of feces (to be discussed later) was considerable, the normal range is given as mean $\pm$ 3 SD. Hence it follows that the upper limit of ^{131}I-excretion is 0.39 per cent.

Expressed as a fraction of total ^{131}I excreted (urine + feces) the fecal ^{131}I constituted up to 1.8 per cent.

Incidentally 3 patients had some diarrhea. This did not place them in the upper part of the ^{131}I-excretion range. The same applied to one patient with blood and another o with trace of blood in feces.

It appears from Table II, where t patients are listed according to their album degradation rate (beginning with the low one), that there was no relation betwe degradation rate and ^{131}I content of stoo

Fecal nitrogen averaged less than 1.7 per day (mean + 2 $\times$ SD).

Hypoproteinemic patients with gastro-intestinal disease.

Group A. 12 patients with *hypercatabo hypoalbuminemia.* All these patients had abnormally high ^{131}I-excretion in the sto (Table III). The highest excretion (1 per cent) was found in a patient with sev ulcerative colitis (No. 5) and in an inf (No. 10) with protein-losing enteropat (more than 6.0 per cent). Both of them h diarrhea.

Except in case No. 5 with ulcerat colitis, no relation was found between and nitrogen content of the stools. Patie with steatorrhea had no specially high excretion.

Fecal ^{131}I-output expressed as fraction the total amount of ^{131}I excreted was nor in 4 of the 12 patients.

In case No. 6 a little more than half

[1] Soybean trypsin inhibitor was supplied by NOVO, Copenhagen.

Table II. *^{131}I-albumin metabolism and fecal ^{131}I-excretion following intravenous ^{131}I-albumin injection in 15 controls.*

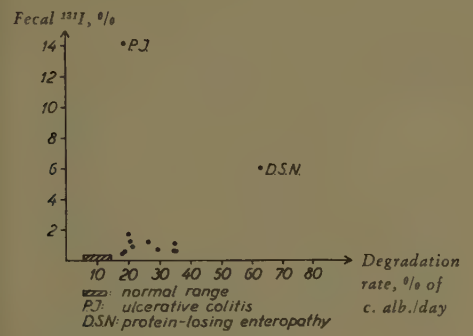
	Age years	Diagnosis	Serum Albumin g/100 ml	Albumin turnover		Fecal ^{131}I-output in 5 days		Diarr.	Fecal content of	
				Relative % of c.alb.*/day	Absolute g/kg/day	% of inj. dose	% of tot. ^{131}I-excret.		Nitrogen g/day	Blood (Benz.)
1. M.H. ♂	76	Duod. ulcer	4.02	4.1	0.11	0.09	0.92	+		—
2. A.N. ♂	43	Duod. ulcer	4.63	7.0	0.17	0.25	1.29	—		—
3. A.J. ♂	53	Duod. ulcer ?	4.58	7.1	0.14	0.01	0.01	—		(+)
4. J.G. ♂	65	Duod. ulcer + obesity	3.84	8.4	0.14	0.05	0.31	—		—
5. F. ♂	50	Disc. degen.	3.80	8.9	0.17	0.16	0.57	—	1.64	—
6. W.T. ♂	44	Duod. ulcer	3.88	9.2	0.20	0.07	0.30	—	1.16	—
7. P.T.C. ♂	18	Gastritis	4.49	9.3	0.20	0.14	0.56	—	1.24	—
8. O.O. ♂	21	Dyspepsia	4.07	9.5	0.20	0.20	0.99	—	1.29	—
9. T.M. ♂	52	Gastr. ulc.	4.29	9.5	0.18	0	0	—		+
10. S.R. ♂	53	Duod. ulcer	3.84	9.8	0.16	0.22	0.90	—		—
11. N.O.A. ♂	23	Duod. ulcer	4.28	11.2	0.19	0.26	0.99	—	0.87	—
12. J.H. ♂	35	Dyspepsia	3.73	11.9	0.16	0.09	0.36	—	0.85	—
13. W.A. ♂	35	Duod. ulcer	4.01	12.3	0.21	0.22	0.85	+	1.36	—
14. O.B. ♂	53	Gastroduod.	4.28	12.5	0.18	0.19	0.99	+	0.83	—
15. K.K. ♂	58	Aur. flutter	4.24	15.7	0.24	0.12	0.41	—	1.04	—
Mean						0.14	0.63		1.14	
SD						0.085	0.392			
Range (Mean ± 3 SD)						0–0.39	0–1.81			
						(n=15)	(n=15)		(n=9)	
Normal average			4.41	10.0	0.206					
Range (Mean + 2SD) (n=20) (Jarnum & Schwartz, 1960)			3.77–5.05	5.6–14.4	0.134–0.278				0.60–1.68	

* C. alb. = Circulating albumin.

Table III. *12 patients with hypercatabolic hypoalbuminemia, gastric (1—4) or intestinal (5—10) disease and abnormal fecal ^{131}I-output.*

	Age	Diagnosis	Serum Albumin g/100 ml	Albumin turnover		Fecal ^{131}I-output in 5 days		Diarr.	Fecal content of			Albumin in gastric-juice
				Relative % of c. alb./day	Absolute g/kg/day	% of inj. dose	% of tot. ^{131}I-excret.		Nitrogen g/day	Fat g/day	Blood (Benz)	
1. E.N. ♂	33	Duod. ulcer + Hypertr. gastr.	2.72	17.9	0.27	0.43	1.30	—	0.50	3.2	+	—*
2. M.C. ♂	57	Hypertr. gastr.	2.58	18.3	0.19	0.45	1.28	—	0.90	2.1	+	+
3. W.D. ♂	72	Gastr. papillom	1.02	19.8	0.09	1.75	5.23	+	1.59		—	+
4. S.T. ♀	63	Ulc. gastrit. + Postgastrect. + Steatorrhea	2.29	20.5	0.24	1.23	2.75	—	1.76	9.9	—	+
5. P.J. ♂	32	Ulcerat. colit. Amyloidosis	1.95	18.4	0.41	14.1	35.6	+	6.60		+	
6. B.P. ♀	49	Unknown intest. disease	1.88	26.2	0.21	1.18	2.78	—	0.86	2.2	—	—
7. R.L. ♂	38	Int. granulom steatorrhea	2.74	29.3	0.43	0.66	1.45	+	2.22	11.2	+—	—
8. E.S. ♀	30	Unknown intest disease	2.45	34.6	0.34	0.58	1.25	(+)	1.76			—*
9. J.C. ♀	49	Int. lymphogran.	1.20	34.7	0.28	1.08		—	3.8			
10. D.S.N. ♀	½	Unknown int. disease	1.69	62.4	0.68	5.97 (2 days)	23.1	+			—	
11. A.J. ♀	40	Postop. after part. gastrect. for ulcer	4.85–3.46	21.2max		0.86	3.57	—	1.06		—	—
12. E.T. ♀	54		4.39–2.96	34.7 —		0.58	2.40	—	0.66		+	+
Normal range			3.77–5.05	5.6–14.4	0.13–0.28	≦0.40	<1.8		<1.7	<6**		

*Gastric juice from cases 1 (E.N.) and 8 (E.S.) was consistently acid (pH < 3) and proteolytic.
**Cooke & French (1958).



ig. 1. The relation between fecal ^{131}I-excretion llowing intravenous ^{131}I-albumin injection and bumin degradation rate in 12 patients with hyper-catabolic hypoalbuminemia.

er cent) of the activity was non-dialysable. No parallellism was found between fecal ^{131}I-output and albumin degradation rate Fig. 1) or serum albumin (Fig. 2). How-ver, it appeared that in all patients with rum albumin below 2.4 g per 100 ml, fecal ^{131}-output was higher than 1 per cent of the jected dose, and in the rest it was lower.

Of the 4 patients with gastric diseases Nos. 1—4) albumin was detectable by paper ectrophoresis of gastric juice in 3. In the urth one (No. 1) gastric juice was con-stently acid and proteolytic, which invalid-ed the electrophoretic tracking.

Group B. 16 patients with gastrointestinal isease and *normocatabolic hypoalbumin-mia.* In 9 patients (Table IV) the fecal ^{131}I-output was normal, in 7 (Table V) it as abnormally high.

Normal ^{131}I-excretion was found in 3 artially gastrectomized patients (including ne with steatorrhea), in one with a gastro-ntero-anastomosis, in 4 with gastric carci-oma and in one previously subjected to assive intestinal resection and now suf-ering from marked steatorrhea.

The series of 7 patients with *abnormal ^{131}I-excretion* included 2 with postgastrectomy syndrome, 3 with hypertrophic gastritis and 2 with regional enteritis. The highest ^{131}I-excretion (4 per cent of the injected dose) was found in case No. 6 (Table V) with regional enteritis. Her degradation rate (8.3 per cent of circulating albumin per day) was below normal mean (10 per cent), and her absolute turnover (0.13 g per kg per day — presumed to equal albumin production) was in the lower part of normal range (0.13 —0.28 g per kg per day) (Jarnum & Schwartz, 1960). In 2 patients (Nos 4 and 5) the fecal fraction of the total amount of ^{131}I excreted was normal.

A special note should be devoted to case No. 5 with hypertrophic gastritis and hypoproteinemic edema. A ^{131}I-PVP test was abnormal. He was subjected to partial gastrectomy. After the operation his hypoproteinemia and edema disappeared, ^{131}I-PVP became normal (Schwartz & Jarnum 1959).

Hypoproteinemic patients without gastrointestinal disorders.

These 8 patients (Table VI) included 4 with a rapid albumin turnover (3 nephrotics

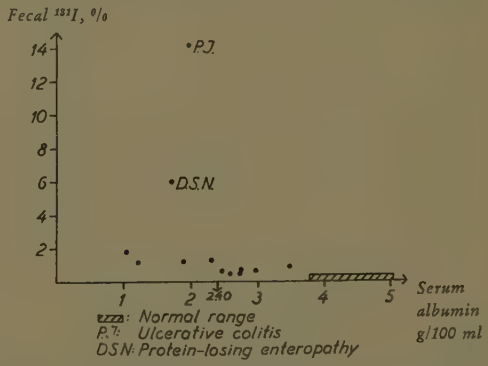


Fig. 2. The relation between fecal ^{131}I and serum albumin concentration in 12 patients with hyper-catabolic hypoalbuminemia.

Table IV. *9 patients with normocatabolic hypoproteinemia, gastrointestinal disease, and normal fecal* ^{131}I*-output.*

	Age	Diagnosis	Serum Albumin g/100 ml	Albumin turnover		Fecal ^{131}I-output in 5 days	
				Relative % of c. alb./day	Absolute g/kg/day	% of inj. dose	% of tot. ^{131}I-excret.
1. G.F. ♀	43	Postgastrect. + macroc. anemia	2.46	6.1	0.12	0.27	1.43
2. F.L. ♂	62	Postgastrect. + steatorrhea	2.95	7.4 (M)*	0.17	0.39	1.99
3. A.A. ♀	73	Gastro-entero-anast. + card. fail.	2.55	8.8	0.14	0.20	0.49
4. C.C. ♂	57	Gastr. carcinoma	2.85	9.7	0.19	0.25	0.90
5. T.I. ♀	28	Hypertr. gastrit + acute tonsillit	3.17	13.9–9.6**	0.16	0.05	0.03
6. C.R. ♀	64	Gastr. carcinoma + chylothorax	3.38	11.5	0.11	0.36	2.04
7. R.C. ♂	69	Gastr. carcinoma	1.48	12.5	0.12	0.23	0.90
8. L.P. ♂	53	Gastr. carcinoma + cutan. metast.	1.87	12.9	0.18	0.26	0.88
9. P.N. ♂	48	Massive intest resection	2.88	10.1	0.19	0.31	1.71
Normal range			3.77–5.05	5.6–14.4	0.13–0.28	<0.40	<1.8

*M: calculated after Matthews (1957).

**Case 5 (T.I.) contracted an acute tonsillitis on the third day of ^{131}I-albumin study. Her degradation rate was on an average 13.9 per cent/day during the period of fever (4 days) and 9.6 per cent/day during the subsequent 6 days.

and one with a pyelonephritis supervening a chronic hepatitis). An abnormal fecal ^{131}I-output was found in only one patient (case No. 8 with nephrosis). His "prerenal" degradation rate, calculated from non-protein-bound urinary activity, is given parenthetically. It was considerably higher than normal. He displayed no signs of gastrointestinal disease, and a ^{131}I-PVP test was normal.

Comparison of fecal ^{131}*I-output after intravenous administration of* ^{131}*I-PVP and* ^{131}*I-albumin.*

The normal ^{131}I-excretion after intravenous injection of ^{131}I-PVP in 22 normoproteinemic control patients has been found to be 0.03—1.01 per cent of the injected do (mean ± 2 SD). The highest value actual found was 1.00 per cent. None of these i cluded patients who served as ^{131}I-albun controls. (Jarnum, 1961).

Comparative studies were done in hypoproteinemic patients .The results a listed in Table VII. The first 14 patie suffered from various gastrointestinal d orders, and 12 of these had an abnorm ^{131}I-excretion after both ^{131}I- albumin a ^{131}I-PVP. 2 patients — No. 7 with a po gastrectomy syndrome and one nephro (No. 15) — displayed a normal ^{131}I-exc tion in both tests. In two patients the te disagreed: In No. 6 R.C. with gastric car

oma, the PVP test was slightly abnormal, t fecal ^{131}I after ^{131}I- albumin was normal. No. 16, J.J. with nephrosis, the adverse sult was found.

The relation between fecal ^{131}I-excretion ter ^{131}I-albumin and ^{131}I-PVP on the one nd and albumin degradation rate on the her hand is graphically demonstrated in g. 3. It appears that a high ^{131}I-excretion ter PVP generally reflected a high degra- tion rate, whereas no such relation was esent after ^{131}I-albumin.

DISCUSSION

The content of radioactivity in a given sample of es homogenate was calculated as the difference tween two counting rates: that of the sample and e background value which was about 110 counts r minute (cpm). Background was counted for 0—) 30 minutes giving a proportional standard ror of (3—) 1.7 per cent. As the net count of veral samples of low activity was given as a all difference between two proportionally high unting rates, the standard error of the net count came considerable. Thus, in two of the 15 con- ol patients (Table II) the fecal activity was less an 10 per cent of background. In the rest of em the net counting rate varied from 25 to 80 r cent of background. The samples were counted r 10 to 30 minutes. The standard error of the net unt (SE_n) was calculated from the formula

$$SE_n = \sqrt{SE_b^2 + SE_s^2}$$

here SE_b and SE_s were standard errors of the ckground and sample respectively (Francis, Mul- gan & Wormall, 1959). A net count of 25 per nt of background would thus give a proportional ndard error of 15 per cent when both background d sample were counted for 10 minutes. This presented the maximum proportional standard ror in 13 of the 15 control values.

Concerning the "hypercatabolic" patients (Table I) a considerable amount of activity was present feces in every case. The net count ranged from 0 to 1350 per cent of background resulting in a oportional standard error which in most cases as appreciably less than 5 per cent.

In "normocatabolic" patients with an abnormal I-excretion (Table V) — a group in which a atistical evaluation was essential — the net count the stools was from 100 to 450 per cent of back- ound. The maximum proportional standard error rived from radioactive measurement was 5.2 per nt. Additional errors arose from collection of sample, weighing etc., but they practically all tend- ed to underestimate the actual fecal ^{131}I-excretion. If this was, say, 0.40 per cent of the injected dose (*i. e.* just above upper normal limit) then the pro- bability that this was a fortuitous deviation from normal mean would still be less than 0.01.

Hence it may concluded that in all cases where an "abnormal" fecal excretion was found, it was significantly above the normal excretion range.

The *in vitro* experiments permitted the conclusion that the breakdown of proteins in feces proceeded at a rather slow rate (about one fourth in 24 hours), but naturally this was only a rough approximation of the real conditions in the colon. However, it might suggest that if an abnormal amount of ^{131}I-albumin passed the ileocoecal valve, then at least part of it could be recovered from the stools.

In the present investigations stool collection was continued sometimes until 7 or 8 days after the injection of ^{131}I-albumin or ^{131}I-PVP, because the passage of carmine might last 2 or 3 days. This long collecting period was chosen first to ascertain that the stools examined might contain the total amount of ^{131}I leaked out in a full 5 days period (even in the stomach), and secondly because the evaluation of daily fecal loss of nitrogen and fat became more accurate the longer the period was.

In the socalled *idiopathic hypoproteinemia,* a syndrome of hypoproteinemia and edema without evidence of renal or hepatic failure, a rapid ("hypercatabolic") turnover of serum albumin as determined by ^{131}I-albumin has been repeatedly observed (Bariéty & Gajdos 1959, Cattell & Norris 1957, Corbeel, Malbrain & Visscher 1954, Gordon 1959, Gross, Embree, Bally, Shipp & Thorn 1960, Holman, Nickel & Sleisenger 1959, Janssen 1957, Schwartz & Jarnum 1959, Schwartz & Thomsen 1957, Thorn, Renold, Froesch

Table V. *7 patients with normocatabolic hypoalbuminemia, gastric (1-5) or intestinal (6-7) disease and abnormal fecal* ^{131}I*-output.*

	Age	Diagnosis	Serum albumin g/100 ml	Albumin turnover		Fecal ^{131}I-output in 5 days	
				Relative % of c. alb./day	Absolute g/kg/day	% of inj. dose	% of tot. ^{131}I-excret.
1. E.J. ♂	49	Postgastrect.	3.38	5.8	0.16	0.55	4.32
2. S.Å.H. ♂	50	Hypertr. gastr.	3.58	6.8	0.16	0.74	3.95
3. R.V. ♀	43	Postgastrect. + steatorrhea	3.57	10.6	0.25	1.71	5.01
4. K.F. ♂	65	Gastric ulc. + hypertr. gastr.	3.16	11.0	0.17	0.48	1.62
5. H.M. ♂	62	Hypertr. gastr.	2.45	13.4*	0.32*	0.47	1.68
5a H.M.		After part. gastrectomy	3.78	8.0	0.16	0.14	0.79
6. S.M. ♀	35	Reg. enterit. steatorrhea	2.70	8.3	0.13	3.99	15.2
7. N.T.K. ♂	19	Reg. enterit.	1.90	13.4	0.15	1.23	2.98
Normal range			3.77–5.05	5.6–14.4	0.13–0.28	<0.40	<1.8

*In case 5 (H.M.) ^{131}I-albumin was given after a period of serum infusions.

& Crabbé 1956). Furthermore, an abnormal gastrointestinal leakage of high molecular substances has been demonstrated in every case examined with ^{131}I-PVP (Gordon 1959, Hsia, White & Hugh-Jones 1959, Martini, Strohmeyer & Bünger 1960, Royer 1960, Schwartz & Jarnum 1959, Vesin, Renault & Cattan 1959, Vesin, Troupel, Acar, Renault, Desbuquois & Cattan 1960, Young, Fowler, Barnett & Miles 1959). Accordingly a proteinleaking lesion of the gastrointestinal tract would be expected in all cases of hypercatabolic hypoalbuminemia without renal or hepatic involvement. This was the reason why the present material of hypoalbuminemic patients with gastrointestinal diseases was subdivided into hyper- and normocatabolic groups.

In Table III it was shown that in the hypercatabolic group all subjects displayed an abnormal fecal ^{131}I-excretion followi intravenous ^{131}I-albumin injection. Eight the 12 patients could be termed idiopath hypoproteinemia as gastrointestinal co plaints were lacking or insignificant. In 6 these a ^{131}I-PVP test was performed. It w abnormal in every case.

In 4 patients (Nos. 1, 5, 11, 12) hyp albuminemia was a secondary finding. only one of these (No. 1) was a PVP t done, which, too, was abnormal. Thus, in of 12 cases an abnormal gastrointestinal pl ma protein leakage had been indirectly monstrated by aid of ^{131}I-PVP, and in remaining 5 patients (Nos. 2, 5, 9, 11, a plasma protein leakage was highly proba owing to the nature of their disease and th hypercatabolic hypoalbuminemia associa with a normal or even increased turnover absolute terms (g per kg per day).

Table VI. *Fecal ^{311}I-output in hypoalbuminemic patients with diseases not affecting the gastrointestinal tract.*

	Age	Diagnosis	Serum albumin g/100 ml	Albumin turnover		Fecal ^{131}I-output in 5 days	
				Relative % of c. alb./day	Absolute g/kg/day	% of inj.dose	% of tot. ^{131}I-excret.
E.N. ♀	73	Myxœdema	2.65	6.4	0.08	0.17	1.23
L.P. ♂	59	Chron. infect.(?) disease, emaciation	2.01	8.5	0.13	0.15	0.69
O.L. ♀	54	Lupus eryth. diss. with proteinuria	2.62	10.3 (8.6)*	0.13	0.02	0–1
M.N. ♂	78	Pyelonephritis + chron. hepatitis	1.60	15.2	0.12	0.10	0.37
J.P. ♂	75	Nephrotic syndr. + cirrhosis	1.09	13.4 (6.5)	0.05	0.07	0.29
S.S. ♂	42	Nephrotic syndr. + Prim. amyloidosis + Postgastrectomy	1.72	23.7 (19.1)	0.21	0.20	0.49
E.H. ♂	49	Nephrosis	1.80	29.5 (11.0)	0.16	0.09	0.14
J.J. ♂	62	Nephrosis	1.09	45.5 (23.8)	0.21	0.45	0.77
Normal range			3.77–5.05	5.6–14.4	0.13–0.28	<0.40	<1.8

1: Myxœdema. 2—4: Chronic infections.
—8: Nephrotic syndromes. *Parenthetical figures denote the degradation rate calculated from non-protein bound urinary ^{131}I-activity.

It appeared that the fecal ^{131}I-excretion hould be given as per cent of the amount njected and not of the total amount excreted ecause, in case of the latter, 4 patients fell nside the normal range. The 2 patients with he highest ^{131}I-excretion (Nos. 5 and 10) ad diarrhea. Otherwise no relation seemed o exist between fecal ^{131}I-output and iarrhea.

The stools from *16 normocatabolic patients with astrointestinal disease* contained an abnormal mount of ^{131}I in 7 cases (Table V). In 5 of these cases 3—7) a PVP test was performed. It was bnormal in 4, but not significantly abnormal in ne (No. 7) who unfortunately had a bad PVP reparation in which almost all ^{131}-I was dialysable. It may be questioned whether an abnormal astrointestinal protein loss could be possible in atients with a degradation rate of serum albumin vhich is lower than the normal mean (Cases 1, 2,). However, as long as there is disagreement re-arding the normal site (or sites) of plasma pro-ein degradation, it is difficult to argue against the possibility of this combination. One of these patients (No. 6) suffered from regional enteritis, a condition in which an abnormal protein loss is well established (Steinfeld, Davidson & Gordon 1957, Steinfeld, Davidson, Gordon & Greene 1960). Actually she displayed an abnormal fecal ^{131}I-excretion after both ^{131}I-albumin and ^{131}I- PVP.

A normal ^{131}I-excretion after ^{131}I-albumin was found in 4 patients with gastric carcinoma (Table IV). This was somewhat surprising, as it has previously been shown that an abnormal plasma protein loss in the stomach is usual in this condition (Jarnum & Schwartz, 1960). In the present series of 4 patients, a PVP test was performed in only one (No. 7). It was slightly abnormal. The albumin degradation rate was above the normal mean — but within the normal range — in three of them.

The stools contained an abnormal ^{131}I-amount in one of 8 *hypoalbumiinemic patients without gastrointestinal disease* (Table VI, No. 8). This was a nephrotic whose "prerenal" albumin degradation rate was considerably higher than normal. This might reflect an abnormal gastrointestinal protein loss, but his PVP test was normal.

In conclusion it can be stated that the fecal ^{131}I-excretion following intravenous in-

Table VII. *^{131}I-excretion after intravenous administration of ^{131}I-polyvinylpyrrolido (^{131}I-PVP) and ^{131}I-albumin to 16 hypoalbuminemic patients.*

	Diagnosis	Fecal ^{131}I-output in 5 days after i. v. injection of	
		^{131}I-PVP	^{131}I-Albumin
1. E.N. ♂	Hypertrophic gastritis + duod. ulcer	1.46	0.43
2. K.F. ♂	Hypertrophic gastritis + gastric ulcer	1.75	0.48
3. H.M. ♂	Hypertrophic gastritis	3.38	0.47
3a H.M. ♂	After partial gastrectomy	1.03	0.14
4. S.T. ♀	Ulcerat. gastritis	4.47	1.23
5. W.D. ♂	Gastric papilloma	3.31	1.75
5a W.D. ♂	After partial gastrectomy	0.16	
6. R.C. ♂	Gastric carcinoma	1.17	0.23
7. G.F. ♀	Postgastrectomy syndr. + steatorrhea	0.36	0.27
8. R.V. ♀	Postgastrectomy syndr. + steatorrhea	1.55	1.71
9. E.S. ♀	Protein-losing enteropathy	4.14	0.58
10. B.P. ♀	Protein-losing enteropathy	5.93	1.18
11. R.L. ♂	Protein-losing enteropathy	7.95	0.66
12. D.S.N. ♀	Protein-losing enteropathy	4.58 (3 days)	5.97 (2 days)
13. N.T.K. ♂	Region. enteritis	1.01*	1.23
14. S.M. ♀	Region. enteritis	2.86	3.99
15. S.S. ♂	Nephrosis	0.11	0.20
16. J.J. ♂	Nephrosis	0.29	0.45
Normal value		<1.02	<0.40

*The ^{131}I-PVP-preparation applied in N.T.K. was of poor quality. Most of the ^{131}I passed a cellopha membrane.

jection of ^{131}I-albumin may be used as a reasonably reliable indicator of an abnormal gastrointestinal protein loss. Thus it was abnormally high in 12 cases of gastrointestinal disease with a hypercatabolic hypoalbuminemia and furthermore in 7 patients with gastrointestinal disease and normocatabolic hypoalbuminemia among whom 5 were shown by other means to suffer from an abnormal gastrointestinal protein loss.

In 2 patients — one with gastric carcinoma (Table IV, No. 7) and one with nephrosis (Table VII, No. 8) — the ^{131}I-PVP test was contradictory.

In the literature the fecal ^{131}I-excretion following ^{131}I-albumin injection has been considered an unreliable indicator of protein leakage, but few data are available. In 1957 Steinfeld, Davidson & Gordon reported th they found from 10 to 50 per cent of intr venously injected ^{131}I (as ^{131}I-albumin) the stools from patients with regional ent ritis and ulcerative colitis as opposed to figure of 0.1 to 1 per cent in patients witho gastrointestinal disease. Recently they ha published further observations on the sa two diseases (Steinfeld, Davidson, Gord & Greene, 1960). In control subjects th found a quite insignificant amount of ^{131}I the stools after intravenous injection of bo ^{131}iodide and ^{131}I-albumin (curiously enou it was almost three times higher aft ^{131}I-albumin). During induced diarrh ($MgSO_4$), fecal ^{131}I loss increased co siderably, but it was still below the upp normal limit given in the present investig

ons. It was confirmed that in regional iteritis and ulcerative colitis the fecal ^{131}I ss may greatly exceed the normal values, id in ulcerative colitis about 50 per cent of e activity was protein-bound.

In a case of idiopathic hypoproteinemia, owler & Miles (1958) found that fecal ^{131}I-xcretion following intravenous ^{131}I-albumin ade up from 1 to 8 per cent of total daily -excretion. Less than 2 per cent of fecal ^{131}I was protein-bound. Later, (Young, Fow-r, Levine & Miles, 1959) an abnormal VP-test was demonstrated in the same itient.

Freeman (1958) studied a man with idio-ithic hypoproteinemia by means of ^{131}I-bumin and ^{131}I-gamma-globulin. He found rapid turnover in either case (about 40 per nt of circulating protein was degraded per 4 hours). Not less than 30 per cent of the tal daily ^{131}I-excretion left the body as rotein-bound ^{131}I in the stools in the ^{131}I-amma-globulin study. Furthermore he de-onstrated protein-bound activity in duo-enal juice. It appears as if Freeman was e first to suggest and bring evidence of a astrointestinal protein leakage in idiophatic ypoproteinemia.

Common in these reports is the fact that l patients investigated suffered from *intest-al* disease. It might be expected that a akage of plasma protein in the gut would sult in part of the protein or its break-own products leaving the body *via* the ools, because the passage through the re-aining intestinal tract would not permit a mplete reabsorption. However, in "gastric rotein losers" it seems strange that a com-ete reabsorption should not take place, pecially when one considers that orally

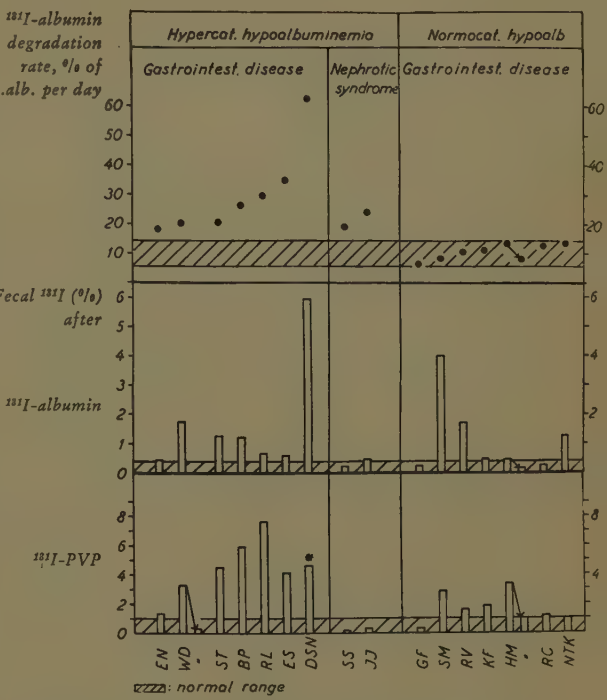

^{131}I-albumin degradation rate, % of c.alb. per day
Hypercat. hypoalbuminemia
Normocat. hypoalb.
Gastrointest. disease
Nephrotic syndrome
Gastrointest. disease
Fecal ^{131}I (%) after ^{131}I-albumin
^{131}I-PVP
EN WD ST BP RL ES DSN SS JJ GF SM RV KF HM RC NTK
normal range

Fig. 3. Fecal ^{131}I-excretion after intravenous injection of ^{131}I-albumin (above) and ^{131}I-polyvinylpyrrolidone (below) in 16 patients with hypercatabolic (left) and normocatabolic (right) hypoalbuminemia.

The arrows denote changes following partial gastrectomy in patients with protein-losing gastropathy (W.D. and H.M.).

The middle two patients (S.S. and J.J.) were nephrotics, all other patients suffered from gastrointestinal diseases.

* The actual ^{131}I-PVP excretion in stools from D.S.N. was considerably higher, as only stools from days 1 and 3 after injection could be collected.

supplied ^{131}I-albumin is almost completely broken down and the ^{131}I absorbed. Nevertheless the stools contained an abnormal amount of ^{131}I following intravenous ^{131}I-albumin in 5 cases of "protein-losing gastropathies" in whom the diagnosis had been confirmed by an abnormal PVP test (Table

VII, Nos. 1—5). In only one case of gastric disease were the two tests discordant (No. 6 with gastric carcinoma).

A certain relationship was found between albumin degradation rate and the amount of fecal ^{131}I following intravenous injection of ^{131}I-PVP, but none after ^{131}I-albumin (Fig. 3). Thus it would appear that a quantitative estimation of gastrointestinal protein loss is permissible — although not very accurate — from fecal ^{131}I after ^{131}I-PVP. In this respect ^{131}I-albumin was useles, but as to the qualitative assessment whether there was abnormal gastrointestinal plasma protein leakage or not, it seemed to be quite efficient. Thus measurement of fecal content of radioactivity is recommended when ^{131}I-albumin degradation studies are undertaken in subjects with hypoproteinemia of undetermined origin.

SUMMARY

The fecal excretion of ^{131}I was determined over a period of 5 days following intravenous injection of ^{131}I-albumin in 51 individuals.

In *15 control patients* with normal serum proteins the average ^{131}I-excretion was 0.14 per cent of the injected dose. The range (mean ± 3 SD) was 0—0.39 per cent. Expressed as a fraction of the total amount of ^{131}I excreted fecal ^{131}I content was 0.63 per cent (range: 0—1.81 per cent).

In *28 patients with hypoalbuminemia and gastrointestinal disease* the fecal ^{131}I-excretion exceeded the normal range in all (12) patients whose albumin degradation rate was above normal (*i. e.* more than 14.4 per cent of the circulating albumin per 24 hours). In the remaining 16 patients, who displayed a normal degradation rate, fecal ^{131}I-output was normal in 9, and abnormal high in
A ^{131}I-PVP test was abnormal in 4 of t
latter 7 subjects.

In *8 hypoalbuminemic patients witho gastrointestinal disease* the fecal ^{131}I-exc tion was normal in 7. A nephrotic had increased excretion.

A *comparison* of ^{131}I-excretion in stools following intravenous injection *of ^{13} PVP and ^{131}I-albumin* in 16 hypoalbumi mic subjects generally showed good agr ment. Twelve patients (all with gastrointe inal disease) had an elevated and 2 a norm ^{131}I-excretion in both tests. In 2 patients test was normal and the other one sligh abnormal. The fecal ^{131}I-content bore no lation to albumin degradation rate af ^{131}I-albumin, but — to some extent — af ^{131}I-PVP.

In *conclusion,* it appeared that a high fe ^{131}I-excretion after intravenous injection ^{131}I-albumin suggested an abnormal plas protein leakage in the gastrointestinal tr Determination of fecal ^{131}I-content sho be included in ^{131}I-albumin degradation s dies of subjects with unexplained hy albuminemia.

REFERENCES

Bariéty, M. & Gajdos, A.: Hypoprotéinémie i pathique. Presse med. *67,* 965, 1959.

Cattell, W. R. & Norris, T. St. M.: Idiopa hypercatabolic hypoprotenemia. Proc. Roy. Med. *50,* 514, 1957.

Cooke, W. T. & French, J. M.: Steatorrhoea. Modern trends in gastroenterology. Se series, p. 218. Butterworth & Co., Lo 1958.

Corbeel, L., Malbrain, H. & Visscher, M. L'hypoprotéinémie essentielle chez l'en Acta pædiat. belg. *8,* 337, 1954.

Fowler, J. F. & Miles, J. M.: Rapid protein br

down in idiopathic hypoproteinaemia. Radioakt. Isot. Klin. Forsch. *3, 298,* 1958.

rancis, G. E., Mulligan, W. & Wormall, A.: Isotopic tracers, p. 210. Second edition. University of London, the Athlone Press 1959.

reeman, T.: In: Radioakt. Isot. Klin. Forsch. *3,* 311, 1958.

ordon, R. S. Jr.: Exudative enteropathy. Abnormal permeability of the gastrointestinal tract demonstrable with labelled polyvinylpyrrolidone. Lancet *1,* 325, 1959.

— Bartter, F. C. & Waldmann, Th.: Idiopathic hypoalbuminemias. Clinical staff conference at the National Institute of Health. Ann. Int. Med. *51,* 553, 1959.

ross, P. A. M., Embree, L. J., Bally, P. Shipp, J. C. & Thorn, G. W.: Hypoalbuminemia (with anasarca) due to hypercatabolism, serum protein exudation into the gastrointestinal tract, increased capillary permeability and hypoanabolism. Am. J. Med. *29,* 386, 1960.

olman, H., Nickel, W. F. Jr. & Sleisenger, M. H.: Hypoproteinemia antedating intestinal lesions, and possibly due to excessive serum protein loss into the intestine. Am. J. Med. *27,* 963, 1959.

sia, D. Y. Y., White, H. & Hugh-Jones, K.: Idiopathic hypoproteinemia. Demonstration of gastrointestinal protein loss through use of I^{131} labelled P.V.P. Clin. Research *7,* 392, 1959.

anssen, B.: Essentiële Hypoproteinaemie. Thesis. Dekker & van de Vegt N. V., Nijmegen 1957.

arnum, S. & Schwartz, M.: Hypoalbuminemia in gastric carcinoma. Gastroenterology *28,* 769, 1960.

— & Schwartz, M.: Studies of albumin catabolism by means of ^{131}I-labelled albumin. Nord. med. *63,* 708, 1960.

aurell, C.-B., Laurell, S. & Skoog, N.: Buffer composition in paper electrophoresis: Considerations on its influence with special reference to the interaction between small ions and proteins. Clin. Chem. *2,* 99, 1956.

artini, G. A., Strohmeyer, G., and Bünger, P.: Exudative Enteropathie ("essentielle Hypoproteinämie") mit rezidivierender Gelbsucht und Verkalkungen im Bauchraum. Deutsch. Med. Wchnsch. *85,* 586, 1960.

Matthews, C. M. E.: The theory of tracer experiments with ^{131}I-labelled plasma proteins. Phys. in Med. Biol. *2,* 36, 1957.

Pearson, J. D., Veall, N. & Vetter, H.: A practical method for plasma albumin turnover studies. Radioakt. Isot. Klin. Forsch. *3,* 290, 1958.

Royer, P.: Hypoprotéinémie chronique idiopathique portant sur les fractions albumine et gammaglobulines, linée a une intolérance au gluten. Ann. Pédiat. (Paris) *36,* 231, 1960.

Schwartz, M. & Jarnum, S.: Gastrointestinal protein loss in idiopathic (hypercatabolic) hypoproteinemia. Lancet *1,* 327, 1959.

— & Thomsen, B.: Idiopathic or hypercatabolic hypoproteinemia. Case examined by ^{131}I-labelled albumin. Brit. med. J. *1,* 14, 1957.

Steinfeld, J. L., Davidson, J. D. & Gordon, R. S.: A mechanism for hypoalbuminemia in patients with ulcerative colitis and regional enteritis. J. Clin. Investigation *36,* 931, 1957.

— Davidson, J. D., Gordon, R. S. Jr. & Greene, F. E.: The mechanism of hypoproteinemia in patients with regional enteritis and ulcerative colitis. Am. J. Med. *29,* 405, 1960.

Thorn, G. W., Renold, A. E., Froesch, E. R. & Crabbé, J.: Pathophysiology of edema. Helvet. med. acta *23,* 334, 1956.

Veall, N., Pearsan, J. D. & Hanley, T.: The preparation of ^{132}I- and ^{131}I-labelled human serum albumin for clinical tracer studies. Brit. J. Radiol. *28,* 633, 1955.

Vesin, P., Renault, H. & Cattan, R.: L'hypoprotéinémie idiopathique. Étude de son mécanisme par le test au polyvinylpyrrolodone-I^{131}. Bull. et mém. Soc. mêd. d. Hôp. de Paris *75,* 319, 1959.

— Troupel, S., Acar, J., Renault, H., Desbuquois, G. & Cattan, R.: Entéropathie avec perte de protéines et stéatorrhée. Étude par le PVP-I^{131} et la trioléine-I^{131}. Action du régime sans gluten. Bull. et mém. Soc. med. d. Hôp. de Paris *76,* 261, 1960.

Young, W. F., Fowler, J. F., Barnett, L. & Miles, J. M.:: Gastrointestinal protein loss in severe malabsorption complicated by idiopathic (hypercatabolic) hypoprotæinemia. Lancet *2,* 85, 1959.

Scandinav. J. Clin. & Lab. Investigation 476—480, 13, 1961.

A METHOD FOR RECORDING RAPID CHANGES IN RADIATION INTENSITY APPLIED TO CARDIOVASCULAR STUDIES

By E. BERNE, L. HALLBERG and S. E. LINDELL

From the Isotope Laboratory, Jubilee Clinic, the Department of Internal Medicine II and the Depart ment of Clinical Physiology, Sahlgren's Hospital, Göteborg, Sweden

(Received for publication February 4, 1961)

In recent years radioactive isotopes have been extensively used in cardiovascular and pulmonary studies to determine for instance cardiac output, pulmonary blood volume or intracardiac shunts.

The determination of the cardiac output by isotopic methods is based on the indicator dilution principle of Stewart and Hamilton. A small amount of a solution containing a known quantity of a suitable radioactive isotope is rapidly injected intravenously. The dilution of the tracer substance in the blood is measured through recording of the momentary activity registered by a detector placed over the heart, over the large arterial blood vessels, or over a catheter through which blood is drawn. The momentary radioactivity over the heart registered by a detector has earlier been recorded with a rate meter and recorder. The time constant of the rate meter has been chosen differently by different authors, the values reported varying between 0.5 (Lammerant 1957, Pietillä & Hakkila 1960) and 2.5 seconds (Huff, Feller, Judd & Borgardus 1955). As the rate of change of the radiation intensity in such measurements may amount to 50 per c per second, or more, it is obvious that dilution process cannot be recorded correc by instruments having the time consta mentioned. However, the use of integrat circuits with an essentially smaller time c stant would result in records too rugged allow accurate calculations. An ideal reco ing method for the study of rapidly chang radiation intensities at a low level should volve actual counting of pulses dur suitable short intervals, under conditi where momentary response to inten changes from one counting interval to next one is ensured. Hence, the interfere between successive counting intervals, typ for the integrating circuits used in meters having long time constants, has to avoided. In cardiovascular studies suita counting intervals would be 0.5—1 second

It is to be noted that the problem can be solved by the use of a counter with pri during the registration of the dilution cess as the operation time of any conceiv printing device is so long that a consider number of the pulses would be lost.

The best way of realizing an ideal count-
g system seems to be the storing of pulses
om the detector in a fast reacting memory
stem where the distance in time between
ccessive pulses is conserved. Using a non-
structive read-out system of the memory
a substantially reduced speed, it is poss-
e to count the pulses within selected count-
g intervals with high precision using ordi-
ry counters. In the present study a record-
g instrument[1] developed by Berne (Berne
Jonsson 1959) was used.

The pulses from a scintillation detector
e recorded on magnetic tape, running at a
gh speed and accepting a pulse rate up to
out 10^6 per minute. In this instrument, the
pe was read out at a speed 16 times lower
an during the recording. The read-out
uld be repeated at will and the pulses were
corded by a counter or by a rate meter.

The purpose of this study was to compare
ution curves obtained with ratemeters with
fferent time constants, and to study the
fluence of the time constant on the calcu-
ion of cardiac output from the dilution
rves. Comparisons were also made with
e dilution curves constructed from the
tivity of arterial blood samples, collected
intervals of 1 second.

METHODS

Dogs were anesthetized with sodium pentabar-
one, and polythene catheters with an internal
meter of 1.6 mm were inserted into both femoral
eries and into the inferior caval vein. One of the
noral catheters was led into a fraction collector,
the other was introduced through the lead shield
of a scintillation detector, wound one turn around its 1 x 1 inch sodium iodide crystal and then connected to a motordriven syringe. The pulses from this detector were recorded on the magnetic tape. 1—20 μC of carrier free ^{131}I as sodium iodide in 1 to 2 ml of saline was injected rapidly into the inferior caval vein. Blood fractions were collected at a rate of one fraction per second. The activity of the blood samples from the fraction collector was measured in an ordinary scintillation well counter. The tape record was analysed in different ways — by a counter and by a rate meter using different time constants.

The blood flow to the fraction collector was found to be about 70 ml per minute, the motor-driven syringe sucked blood at a rate of 75 ml per minute. During recording the tape was run at a speed of 60 inches per second. During read-out of the tape with the rate meter the original speed was used, but when the record was analysed with counter, the speed of the tape was reduced to 3.75 inches per second. The counting intervals were 12 sec. corresponding to 0.75 sec. in the original record. The rate meter time constants were 0.3 and 1 second respectively. For the calibration of the scintillation detector, a suitable dilution of the injection solution was sucked through the catheter and the activity measured.

The blood samples from the fraction collector were well shaken and 1 ml of whole blood counted in the scintillation well detector. These samples were counted to 1 per cent accuracy. The well detector was calibrated with a suitable dilution of the injection solution.

CALCULATION AND RESULTS

All recorded data were expressed in μC ^{131}I per liter blood. Figs. 1 and 2 show the dilution curves of experiment I as recorded with counter and rate meter respectively, with the two different time constants. Fig. 3 shows the corresponding dilution curve obtained with a counter together with the same curve as obtained from the blood samples collected by the fraction ollector. The cardiac

[1] This instrument, the Memonucleograph, is
oduced by Nukleonikinstrument AB, Göteborg,
eden.

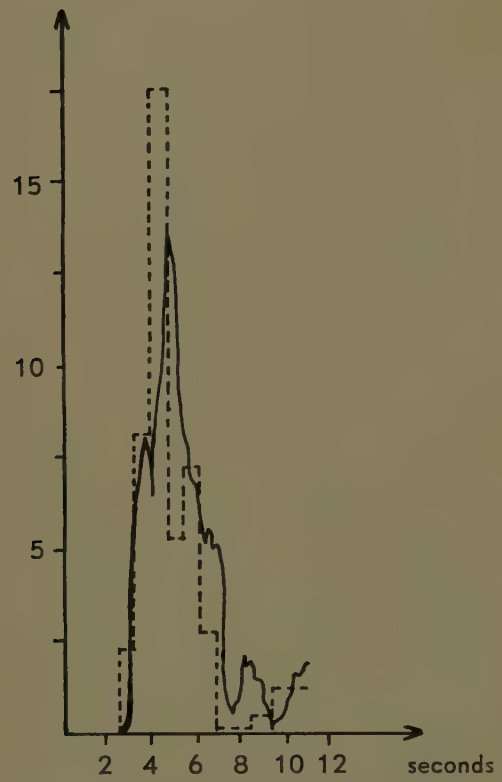


Fig. 1. Dilution curves reproduced from tape recorded by counter - - - - - - - -, by rate meter with time constant 0.3 seconds ————.

output (F) was calculated according to the well-known equation.

$$F = \frac{Q}{\int_{t=0}^{\infty} c(t)\,dt} \qquad (1)$$

where Q = total amount of tracer injected, c(t) = concentration of ^{131}I in the blood without recirculation. For results obtained from fraction collection and from read-out of the tape record with a counter, the integral in the denominator was calculated in the following way: The concentration values obtained were plotted against time in a se logarithmic diagran, the "dilution consta (λ) calculated from the linear part of t curve, and the instant of recirculation det mined as the point where the logarith dependence of the concentration on ti ceased. The integral was then calcula according to the formula:

$$\int_{t=0}^{\infty} c(t)\,dt = \sum_{i=1}^{r} c_i \cdot \Delta t_i + \frac{c_r}{\lambda}$$

where c_i = mean concentration in the ti interval Δt_i,
r = last counting interval before circulation,
c_r = concentration in interval r.

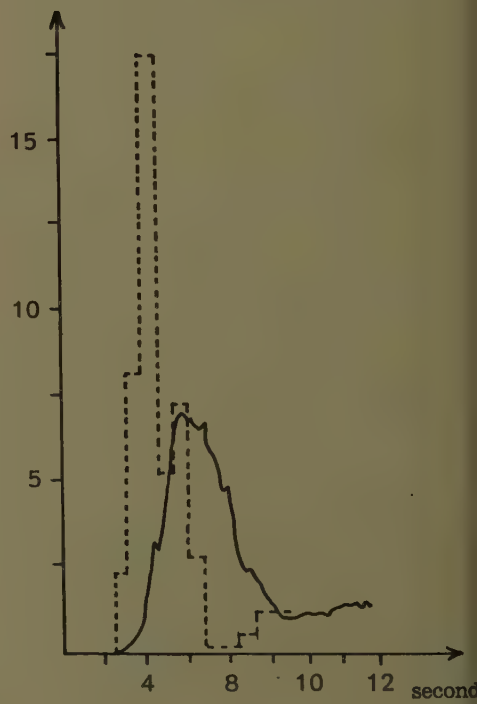


Fig. 2. Dilution curves reproduced from tap counter - - - - - - - -, by rate meter with time stant 1 second ————.

ig. 3. Dilution curves: fraction collector ———, tape recorded read-out by counter - - - - - -.

or the results obtained by the rate meter om the tape record the integral in the de-ominator of (1) was calculated in the rdinary way through logarithmic extra-olation to zero concentration of the dilution urve from the instant of recirculation ob-ined from the corresponding curve, fol-wed by measurement of the area enclosed y the curve.

The cardiac output determinations in the vo experiments as obtained with the differ-nt methods are shown in Table I.

DISCUSSION

The dilution curve as registered by fractional collection of blood samples is assumed to represent a correct reference. Good agreement was found between the curves obtained from counting of the blood samples in the fraction collector and the curves obtained from interval counting of the tape record. However, the rate meter curves were more or less incorrect. It is evident from Figs. 1 and 2 that, with a longer time constant, the maximum of the curve is reached later, the maximum value is lower, and the curve is more distorted the longer the time constant. It is also evident that the effect of the recirculation on the dilution curve is more difficult to observe the longer the time constant. Besides the distortion of the dilution curve, which makes impossible a correct analysis of the concentration as a function of time, a finite time constant tends to make the area under the dilution curve too small during the rise of the curve, *i. e.* an error which makes the cardiac output figure too high is introduced in this part. During the fall of the curve, the area tends to become too large,

Experiment	Cardinac Output			
	A	B.	C	D
I	2.0	1.8	1.6	1.9
II	1.17	1.20	1.20	1.10

Table I. Cardiac output (liters per minute) as calculated from: A) Blood samples collected at intervals of 1 second, B) dilution curve recorded with a counter from tape record C) dilution curve recorded with a rate meter with time constant 0.3 second D) rate meter record with time constant 1 second. In experiment I 1 μC ^{131}I was injected, in experiment II 20 μC ^{131}I.

thus introducing an error, which makes the output value too low. With a given time constant, the positive and negative errors may sometimes cancel. This is, however, quite fortuitous and depends on the chosen time constant and on the shape of the dilution curve, this shape depending on *i. a.* the rate of injection, the cardiac output, and on the size of the mixing chamber. This is illustrated by the results shown in Table I. In experiment I, where only 1 μC was injected the total number of counts in the recording with a counter were only 50, giving a statistical uncertainty of 14 per cent, the agreement between the results of the analysis of blood samples and the results obtained through read-out of the tape record with a counter is within the statistical counting accuracy. It is found, however, that the results obtained after read-out with a rate meter give a correct value, in experiment I, at a time constant of 1 second while in experiment II a time constant of 0.3 seconds is needed to give a correct value.

From the examples, it is evident that, in cardiac output determinations with external detectors, the use of a fast memorizing instrument as in this study is necessary to obtain correct dilution curves. An other advantage of such a system is that the record can be reproduced at will and that it may be analysed at slow speed allowing a 16 fold stretching of the time axis. Repeated read-out with different counting intervals often enables a better analysis of the investigated courses.

In general, this type of recording with inertia should be valuable in the study of phenomena where radiation intensities v rapidly, especially at low intensities.

SUMMARY

A new method including the registrat of the pulses from a scintillation detector magnetic tape has been applied to recording of dilution curves in the bl after administration of radioactive iodide.

The experiments indicate that the dist tion resulting when such dilution curves registered by rate meters is avoided, so t a correct reproduction of the radioacti in the blood as a function of time is m possible.

This type of recording should be of va in all case where rapid changes in radiat intensities are studied.

REFERENCES

Berne, E. & Jonsson, U.: Ein magnetischer A lysator für die quantitative Auswertung Scintigrammen. Nuclear Medizin *1,* 80, 195

Huff, R. L., Feller, D. D., Judd, O. J. & Bogar G. M.: Cardiac output of men and dogs m ured by in vivo analysis of iodinated ^{131}I man serum albumin. Circulation Researc 564, 1955.

Lammerant, J.: Le Volume Sanguin des Pou Edition Arscia Bruxelles 1957.

Pietillä, K. A. & Hakkila, J.: Studies of car output and of pulmonary and intra-thor blood volume. Cardiologia *36,* 97, 1960.

ndinav. J. Clin. & Lab. Investigation 481—488, 13, 1961.

HYPOKALEMIC ALKALOSIS WITH PARADOXICAL ACIDURIA DURING ARTIFICIAL VENTILATION OF PATIENTS WITH PULMONARY INSUFFICIENCY AND HIGH PLASMA BICARBONATE CONCENTRATION

By H. E. REFSUM

From the University Institute for Respiratory Physiology, Department III, Ullevål Hospital, Oslo, Norway.

(Received for publication December 20, 1960)

In pulmonary insufficiency the increase of e arterial carbon dioxide tension, together th the decrease of the oxygen tension, is expression of the failure in alveolar seous exchange, while the increase of the arbonate concentration partly depends on e renal compensatory mechanisms, which event major pH changes. In gradually in-easing pulmonary insufficiency practically oportional increases of carbon dioxide ten-n and bicarbonate concentrations may be ind, and thus, even in high degrees of pul-onary insufficiency the pH of plasma may normal or practically normal (Peters & an Slyke, 1932; Refsum, 1960).

Since its introduction by Boutourlong-oung & Whittenberger (1951) artificial ntilation is increasingly used in the treat-nt of patients with chronic pulmonary in-fficiency. Blood gas indications for arti-al ventilation have been discussed, among ers, by Erikson & Refsum (1956), Ref-n (1960) and Semb, Erikson & Refsum 961). As to the performance of the artifical ventilation the main interest has been concentrated on the restoration toward normal of the carbon dioxide tension and the oxygen tension, while little attention has been given to the normalization of the bicarbonate concentration.

The purpose of this paper is to present three case reports, which demonstrate how the normalization of the plasma bicarbonate concentration can be delayed in relation to the normalization of the carbon dioxide tension, and to discuss some explanations and consider therapeutic consequences.

METHODS

Usually the blood samples were collected at eight o'clock in the morning, before the patients received any food. The sampling of the arterial blood and the determinations of the arterial blood gas values were performed as described previously (Refsum, 1960). The samples taken during artificial ventilation were taken through the shutters of the tank respirator, without interfering with frequency and pressures.

Table I. *Blood gas and electrolyte observations in case I (G. B. 53 years). For expla tion, see text. (S: Spontaneous ventilation. R: Continuous artificial ventilation. A: Arte sample. V: Venous sample.)*

Date	20/10	31/10	9/11	2/12	16/12		19/12	23/12	29/12	5/1	18/1	1
					1200	2200						
No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(
HbO_2, %	71.0		55.0[1])		91.0	93.0		82.0		88.0	89.0	8
TCO_2, mEq/l	37.0	30.5	41.0	28.0	38.0	39.0	45.0	44.0	36.0	33.0	29.0	3
pCO_2, mm Hg	67		92		40	36		55		50	45	4
pH	7.34		7.25		7.58	7.66		7.51		7.43	7.41	7
K^+, mEq/l	4.3	4.9				2.7	2.7	2.6	5.2	4.5		4
Cl^-, mEq/l	94					80	85	86	92	98		9
Remark	S A	S V	S A	S V	R A	R A	R V	R A	R V	R A	R A	S

[1]) Oxygen through nasal catheter.

In case 3 the urine was collected through an indwelling catheter, and stored in stoppered bottles, with some toluene to prevent bacterial growth.

The concentrations of sodium and potassium in serum and urine were determined with an EEL flame photometer, and the total nitrogen excretion in the urine was determined by micro-Kjeldahl (Peters & Van Slyke, 1932). These analyses were performed at the Central Laboratory, Ullevål Hospital, and the results were kindly placed at the author's disposal.

The concentrations of chloride in serum and urine were determined according to Schales & Schales (1941). The pH of urine was usually determined by means of indicator paper, but frequently checked by means of a Doran Precision Universal pH-Meter.

Usually the artificial ventilation was interrupted, and the patient allowed to sit up for meals for half an hour three to four times daily.

Abbreviations: Hemoglobin oxygen saturation: HbO_2; carbon dioxide content of plasma TCO_2; carbon dioxide tension: pCO_2.

CASE REPORTS

Case 1 (G. B.) was a 53-year-old woman. In 1951 she had a 6-rib thoracoplasty, for pulmonary tuberculosis with empyema. Later her cardiopulmonary function, as judged by the carbon oxide recovery time (Erikson, 1952), has reduced practically to the minimum compa with life. Thus, in connection with an acute cat al infection in the autumn 1957 she developed s pulmonary insufficiency with carbon dioxide and had to be treated continuously, for weeks, tank respirator. Thereby the venous plasma ca dioxide content decreased from 43 to 28 mEq

At the end of October 1959 she was again mitted to Department III, Ullevål Hospital, pulmonary insufficiency. Table I shows some gas observations made during the treatment.

Just after admission there was a marked oxemia and hypercapnia, but a moderate pH r tion (1). During treatment in a tank respira gradual improvement took place (2). The p however, got broncho-pneumonia; in the cour this she was confused and restless and sh severe hypoxemia, hypercapnia and acidosis but by means of continuous artificial ventilat was again possible to reduce the venous p carbon dioxide content to practically normal v (4).

But the pulmonary insufficiency increased For some days it was impossible to ventilat adequately. Only after adjusting the pressur the tank to —28 and +6 cm H_2O and the freq to 28, was the cyanosis relieved and improv

erved clinically. But as the venous plasma car- dioxide content remained high (40 mEq/1), arterial sample was taken. This showed normal on dioxide tension and a slightly subnormal oglobin oxygen saturation; the plasma carbon ide content and pH, however, were markedly eased (5). After artificial ventilation at the e frequency and pressures for an additional ten rs, the carbon dioxide tension was even lower, the hemoglobin oxygen saturation higher; the ma carbon dioxide content, however, was un- red, and the pH definitely higher (6). There a definite hypokalemia and hypochloremia; the a and creatinine concentrations of the plasma e 120 and 2.0 mg per cent respectively, and the e was acid.

The patient was treated with increased admin- ation of water and electrolytes. — Already after ay the plasma concentrations of urea and crea- ne were practically normalized, and thereafter, ing the following weeks, with a daily diuresis of —1500 ml, they remained below 40 and 1.0 mg cent respectively. But in spite of an additional inistration of 30—40 mEq potassium chloride day in six days (together with sodium chloride), potassium concentration of the serum remained (7,8). — The respirator pressures were reduced order to increase the carbon dioxide tension, and reby reduce the alkalosis.

An arterial sample taken six days after the detection of the alkalosis showed that the reduction of the respirator pressures and the subsequent increase of the carbon dioxide tension had led to the intended effect on the pH, which was markedly reduced, but still elevated, and to a further increase of the plasma carbon dioxide content.

Maintaining the same respirator adjustment, and with further increased administration of potassium chloride, now avoiding sodium chloride, the serum potassium concentration increased and the plasma carbon dioxide content decreased synchronously (9); and now, by increasing the respirator pressures the alveolar gaseous exchange could be improved without alkalosis (10, 11). — The plasma chloride concentration varied inversely with the bicarbonate concentration.

The patient was discharged from the hospital with a slight hypercapnia and hypoxemia (12), — nearly four months after admission.

Case 2. (I. K.) was a 60-year-old woman. In 1934 she had a 6-rib thoracoplasty for pulmonary tuberculosis. Early in January 1960 she was admitted to Department III, Ullevål Hospital, with an abscess in the scar from the thoracoplasty, and pulmonary insufficiency. — Table II shows some observations made during the treatment of the patient.

In the course of two weeks the patient got in-

ble II. *Blood gas and electrolyte observations in case 2 (I. K. 60 years). For explana- n, see text. (S: Spontaneous ventilation. R: Artficial ventilation. A: Arterial sample. V: Venous sample.)*

Date	4/1	11/1	17/1	18/1	19/1	20/1	21/1	22/1	23/1	27/2
No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
O_2, %				86.0	88.0				91.5	92.0
O_2, mEq/l	33.0	35.0	43.0	38.0	36.0	38.0	34.0	31.0	28.0	28.5
O_2, mm Hg				47	42				42	45
				7.52	7.54				7.43	7.40
, mEq/l				3.3	3.1			5.4	5.1	4.5
, mEq/l				86	88	89		94	97	102
mark	S V	S V	S V	R A	R A	R V	R V	R V	R A	S A

creasingly cyanotic, and the venous plasma carbon dioxide content increased gradually (1, 2, 3). When the plasma carbon dioxide content had risen to 43 mEq/l (3), the patient was confused and restless, and she was placed in a tank respirator. — Approximately twelve hours later an arterial sample showed an almost normal carbon dioxide tension and a moderately reduced hemoglobin oxygen saturation; the plasma carbon dioxide content, however, was still markedly elevated, and correspondingly the pH was high (4). The serum potassium concentration was slightly reduced, and the urine was acid. The following day, after further increase of the respirator pressures, the carbon dioxide tension was normal and the hemoglobin oxygen saturation was increased. The plasma carbon dioxide content, however, was only slightly reduced, and the pH was further increased. The serum potassium concentration was lower (5). — As the plasma carbon dioxide content was unaltered the next day also (6), the patient on this and the following day was given approximately 40 mEq potassium chloride, whereof 25 mEq intravenously, per day. Maintaining the same respirator adjustment the serum concentration of potassium and the plasma carbon dioxide content were normalized (8, 9), and after six days of respirator treatment, the values for carbon dioxide and pH were quite normal (9). The patient left the hospital with normal carbon dioxide tension, and a slightly reduced hemoglobin oxygen saturation (10).

The plasma concentrations of urea and creatinine remained below 30 and 0.9 mg per cent respectively the whole time.

Case 3. (O. Ø.) was a 59-year-old man. From 1945 he suffered from frequent attacks of bronchitis, and in the course of 1959 he had broncho-pneumonia three times. At the end of February 1960 he was admitted to Ullevål Hospital for right heart failure. The electrocardiogram showed right bundle branch block, and the chest X-ray demonstrated several cavities and widespread infiltrations with shrinkage of the right superior lobe.

One week after admission the temperature rose to 39.5° C, the patient was increasingly dyspnoeic, and in the course of a few hours he went comatous with subsequent respiratory and cardiac arr After immediate intubation, bronchial suction manual ventilation with oxygen, the heart act started again. The patient was placed in a t respirator with oxygen administration throug nasal catheter, and regained consciousness a ventilation for two hours. Fig. 1 shows some servations during the treatment.

The first blood gas determination, performed before the respiratory arrest, demonstrated m kedly increased carbon dioxide tension and redu pH, moderately increased plasma carbon dio content, and severely reduced hemoglobin oxy saturation. The next sample, taken approxima three hours after the start of the respirator tr ment and shortly after the patient had regai consciousness, showed moderately increased ca dioxide values, normal pH and almost nor hemoglobin oxygen saturation. Maintaining respirator adjustment (frequency 24, pressures and + 6 cm H_2O) and the oxygen administrat the blood gas values remained fairly constant ing the next days. Then, since ample pus produc necessitated frequent bronchial suctions, the pa was tracheotomized and taken out of the respira In the following days, while the patient was in bed with intermittent oxygen administration, carbon dioxide values increased gradually. W the carbon dioxide tension had risen to 62 mm and the plasma carbon dioxide content to mEq/l, continuous artificial ventilation in a respirator was again instituted.

After artificial ventilation for approxim sixteen hours the carbon dioxide tension was duced to the upper limit of the normal range; using approximately the same respirator adjust (frequency 22, pressures —24 and +4 cm H it was possible to maintain this carbon dio tension and hemoglobin oxygen saturation du the further course of the treatment. — The pl carbon dioxide content of the samples taken o first and second day after the start of the res tor treatment, however, were almost unaltered correspondingly the pH markedly increased. A same time the serum potassium concentration reduced; the urine was maximally acid, as b the start of the artificial ventilation. The pa

as then given approximately 70—80 mEq potas-
um chloride, whereof 50 mEq intravenously, per
y in four days. With marked positive potassium
lance a simultaneous decrease of the plasma
rbon dioxide content and pH, and increase of
e serum potassium concentration and pH of the
ine took place.

After the cessation of potassium administration,
nen the serum potassium concentration was
rmalized, the urinary sodium excretion (which
d been low) increased markedly, without in-
eased sodium administration, and the pH of the
ine increased further; the serum sodium con-
ntration decreased from 140 to 136 mEq/l. — In
e following weeks, with practically the same in-
ke of food, the potassium excretion in urine av-
aged approximately 50 mEq per day, and the
dium/potassium ratio in the urine was higher
an 2, with few exceptions.

The total nitrogen output in the urine per day
as essentially constant before, during and after
e period with respirator treatment, alkalosis and
lministration of potassium chloride, approximately
0—10.0 g per day.

Except for the first sample after the respiratory
rrest, when the plasma creatinine concentration
as 1.7 mg per cent, all creatinine values were
low 0.9 mg per cent, and the plasma urea con-
ntration remained below 40 mg per cent. With
average water intake of 2500 ml per day the
ily urine output varied between 400 and 1200 ml,
ith the lowest values during the alkalosis. The
ecific gravity of the daily urine was above 1022
e entire time.

The three cases described had severe pul-
onary disease with a high plasma carbon
ioxide content. During continuous artificial
entilation the carbon dioxide tension rapidly
ecreased to normal, or practically normal
alues, but the plasma carbon dioxide con-
nt remained high; the patients thus de-
onstrated, in varying degree, blood gas and
ectrolyte values as in hypokalemic alkalosis
ith paradoxical aciduria. During continued

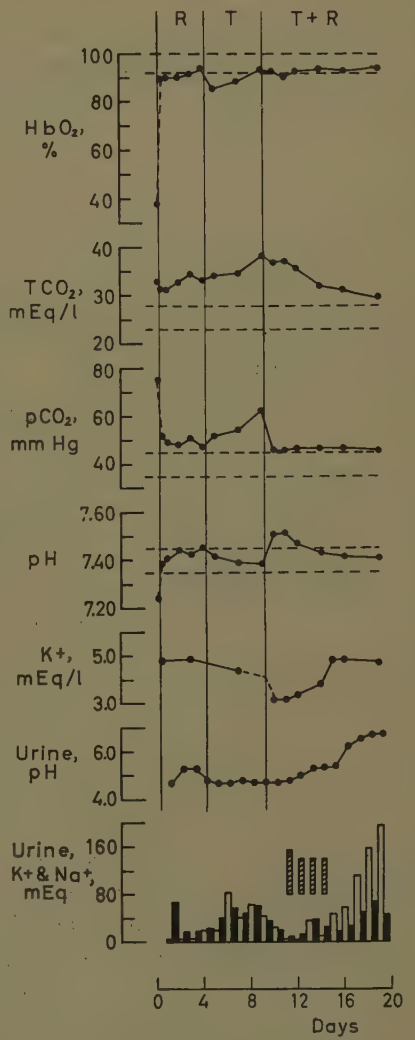


Fig. 1. Arterial blood gas and electrolyte observations in case 3 (O. Ø. 59 years). R: Continuous artificial ventilation with additional oxygen through nasal catheter. T: Spontaneous ventilation with additional oxygen through tracheostoma. T + R: Continuous artificial ventilation with additional oxygen through tracheostoma. The additional oxygen administration, 2 l per minute, was continuous during R and T + R, intermittent during T. In the graph the white bars represent the sodium excretion and the black the potassium excretion. The four cross-hatched bars represent the potassium administration on these particular days.

artificial ventilation and administration of potassium chloride the serum potassium, plasma carbon dioxide content and pH normalized simultaneously.

DISCUSSION

When calculating the plasma bicarbonate concentration and assuming average buffer capacities for the actual blood samples (Henderson, 1928; Davenport, 1950) it appears for all three patients that the base excess remained practically constant from the last sample before the respirator treatment was started until administration of potassium chloride led to increasing serum potassium values. This together with the marked increase in the sodium excretion and the pH in the urine, subsequent to the normalization of the serum potassium in case 3, indicates that the maintenance of the hypokalemic alkalosis with paradoxical aciduria may be explained partly in accordance with present theories for this syndrome; *i. e.* that a low potassium concentration in the tubular cells leads to increased reabsorption of sodium and bicarbonate to the blood, and to increased excretion of hydrogen ions in the urine (Berliner, Kennedy & Orloff, 1951; Roberts, Randall, Sanders & Hood, 1955; Roberts, Randall, Vanamee & Poppel, 1956; Moore, 1959).

Usually the syndrome of hypokalemic alkalosis with paradoxical aciduria results from extraordinary loss of hydrochloric acid and potassium in excess of nitrogen, as for instance due to vomiting (Gamble & Ross, 1924—25; Hartman & Smythe, 1926; Van Slyke & Evans, 1947), or it may be contributed to by aldosterone after surgery or other severe stress (Llaurado, 1955; Bartter, 195 Moore, 1959). — In the present cases it h appeared in connection with relatively acu reduction of the arterial carbon dioxide te sion in patients with compensated respirato acidosis, and therefore it seemes reasonab to interpret the syndrome as an expressi of a delayed normalization of the renal co pensatory mechanisms, originally induced the increased plasma carbon dioxide tensi (Relman, Etsen & Schwartz, 1953; Sulliv & Dorman, 1955). Assuming that potassiu is of major importance for the maintenan of the high plasma bicarbonate concentrati in these cases, the question arises whether t syndrome is due to a previously develop potassium deficiency, or to a hypokalem which is secondary to the extracellular alk losis, and induced by the acute reduction the carbon dioxide tension (Scribner & Bu nell, 1956).

During the development of the respirato insufficiency, prior to the respirator tre ment and the alkalosis, case 1 lost water a food through a broncho-oesophageal fistu In case 2 there were no signs of defici intake or abnormal loss of electrolytes duri the development of the respiratory insuffi ency. Case 3 had transient gastric dilatati with vomiting and diarrhea on the seco day of the first period of respirator tre ment, *i. e.* eight days before the appeara of the alkalosis and the hypokalemia; th however, had no immediate effect on plasma concentrations of potassium chlorides. During the last week before alkalosis there were no clinical signs deficient intake or abnormal loss of elect lytes; the potassium and total nitrogen cretion in urine for the time the patient

eated outside the respirator and the weeks ter the establishment of normal acid-base ate were essentially of the same magnitude. ince the food intake was rather constant, it ppears that no important loss of potassium excess of nitrogen in the urine can have ken place just before the appearance of the ypokalemia and the alkalosis.

Thus, it seems that a potassium depletion ight have taken place during the develop- ent of the respiratory insufficiency in case but probably not in cases 2 and 3. — When rrecting the serum potassium concentra- ons during the alkalosis for the effect of the lasma pH (Scribner & Burnell, 1956; aton, 1960), the values in cases 2 and 3 are ithin the normal range.

Consequently, it seems, at least for cases and 3, that the delay of the normalization the base excess might have been due to a ypokalemia induced by the acute reduction the carbon dioxide tension and the sub- equent increase in the extracellular pH.

If so, it might seem reasonable, in similar ases, to administer acidifying solutes, *e. g.* mmonia chloride, in order to obtain a re- istribution of the body potassium (Scrib- er, Fremont-Smith & Burnell, 1955). The ct, however, that the normalization of the se excess in case 3 took place during a arked positive potassium balance, indicates at the potassium administration not only d a direct effect on the tubular sodium and carbonate reabsorption and the hydrogen n excretion, but also covered an intracellu- r deficiency.

Whatever the explanation might be, the ported data seem to indicate that potas- um administration is of benefit in the treat- ent of hypokalemic alkalosis with para- doxical acidura, appearing during artificial ventilation of patients with pulmonary in- sufficiency and high plasma bicarbonate con- centration.

SUMMARY

Three cases with advanced pulmonary disease and markedly elevated plasma carbon dioxide content were treated with continuous artificial ventilation. After the start of arti- ficial ventilation the arterial carbon dioxide tensions were soon reduced to normal or practically normal values, but the plasma car- bon dioxide contents remained high; and the patients demonstrated in varying degree blood gas and electrolyte values as in hypo- kalemic alkalosis with paradoxical aciduria. Only after administration of potassium chlo- ride (during maintained artificial ventila- tion) did a simultaneous normalization of the serum potassium, plasma carbon dioxide content and pH take place.

The data obtained seem to indicate that administration of potassium is of benefit in the treatment of hypokalemic alkalosis with paradoxical aciduria, appearing during arti- ficial ventilation of patients with pulmonary insufficiency and high plasma carbon dioxide content, — partly to influence the renal re- absorption of bicarbonate and sodium and excretion of hydrogen ions, partly to cover a deficiency in the body.

ACKNOWLEDGMENT

The author wishes to express his thanks to Dr. D. D. Van Slyke for his courtesy in discusing the observations and reading the manuscript.

REFERENCES

Bartter, F. C.: The role of aldosterone in normal homeostasis and in certain disease states. Metabolism *5*, 369, 1956.

Berliner, R. W., Kennedy, T. J., Jr. & Orloff, J.: Relationship between acidification of the urine and potassium metabolism. Am. J. Med. *11,* 274, 1951.

Boutourlong—Young, H. J. & Whittenberger, J. L.: The use of artificial respiration in pulmonary emphysema accompanied by high carbon dioxide levels. J. Clin. Investigation *30,* 838, 1951.

Davenport, H. W.: The ABC of acid — base chemistry. 3rd. ed. University of Chicago Press. Chicago 1950.

Erikson, H.: Spirometry during a short exercise test for evalution of pulmonary function. Scandinav. J. Clin. & Lab. Investigation *4,* 338, 1952.

— & Refsum, H. E.: The carbon dioxide combining power of plasma as an indicator of respiratory insufficiency in the postoperative phase of thoracic surgery. J. Oslo City Hosp. *6,* 161, 1956.

Gamble, J. L. & Ross, S. G.: The factors in dehydration following pyloric obstruction. J. Clin. Investigation *1,* 403, 1924—25.

Hartman, A. F. & Smythe, F. S.: Chemical changes in the body occuring as a result of vomiting. Am. J. Dis. Child. *32,* 1, 1926.

Henderson, L. J.: Blood. Yale University Press. 1928.

Llaurado, J. G.: Increased excretion of aldosterone immediately after operation. Lancet *1,* 1295, 1955.

Moore, F. D.: Metabolic care of the surgical patient. W. B. Saunders Company. Philadelphia and London 1959, p. 383, 389.

Paton, R. R.: Relation between serum potassium and potassium depletion. Clin. Research *8,* 231, 1960.

Peters, J. P. & Van Slyke, D. D.: Quantitative clinical chemistry. Vol. I. Interpretations. Baillière, Tindall & Cox. London 1932, p. 8—1018.

Peters, J. P. & Van Slyke, D. D.: Quantitati clinical chemistry. Vol. II. Methods. Baillie Tindall & Cox. London 1932.

Refsum, H. E.: Arterial blood gases in respirato insufficiency. Acta. chir. Scandinav. Sup *253,* 175, 1960.

Relman, A. S., Etsen, B. & Schwartz, W. B.: T regulation of renal bicarbonate reabsorption plasma carbon dioxide tension. J. Clin. Inves gation *32,* 972, 1953.

Roberts, K. E., Randall, H. T., Sanders, H. L. Hood, M.: Effects of potassium on renal t bular reabsorption of bicarbonate. J. Clin. I vestigation *34,* 666 1955.

— Randall, H. T., Vanamee, P. & Poppell, J. W Renal mechanisms involved in bicarbonate a sorption. Metabolism *5,* 404, 1956.

Semb, C., Erikson, H. & Refsum, H. E.: Ten ye experience with tank respirators in the tre ment of post-operative respiiratory failure. A chir. Scandinav. In print 1961.

Schales, O. & Schales, S. S.: A simple and accur method for determination of chloride in bi ogical fluids. J. Biol. Chem. *140,* 879, 1941.

Scribner, B. H. & Burnell, J. M.: Interpretati of the serum potassium concentration. Me bolism *5,* 468, 1956.

— Fremont-Smith, K. & Burnell, J. M.: T effect of acute respiratory acidosis on internal equilibrium of potassium. J. Clin. vestigation *34,* 1276, 1955.

Sullivan, W. J. & Dorman, P. J.: The renal sponse to chronic respiratory acidosis. J. C Investigation *34,* 268, 1955.

Van Slyke, K. K. & Evans, E. I.: The paradox aciduria in the presence of alkalosis caused hypochloremia. Ann. Surg. *126,* 545, 1947.

Scandinav. J. Clin. & Lab. Investigation 489—502, 13; 1961.

ESTIMATION OF THE SPLANCHNIC BLOOD FLOW IN MAN BY THE BROMSULPHALEIN (BSP) METHOD; THE EFFECT OF CONTINUOUS VARIATION OF THE PERIPHERAL PLASMA CONCENTRATION OF BSP[1]

By H. CASTENFORS, H. ELIASCH and E. HULTMAN

From the Central Laboratory, the 2nd Medical Service and the Roentgen Department, S:t Erik's Hospital and the Department of Medicine, the Serafimer Hospital, Stockholm, Sweden.

(Received for publication February 6, 1961)

To estimate the splanchnic blood flow (ESBF) by the bromsulphalein (BSP) method, two values must be determined:

1. The difference between the arterial and the hepatic venous plasma concentration of BSP.

2. The rate at which the liver removes BSP from the blood.

While the plasma concentration can be measured directly, the rate of removal can only be determined indirectly by assuming that it equals the rate at which BSP is infused. This assumption is valid only when the plasma concentration is constant, but minor deviations are of little importance since appropriate corrections can be made (Bradley, Ingelfinger, Bradley & Curry 1945). Such corrections are believed to be less effective in the face of major fluctuations of the BSP concentration as sometimes encountered when the splanchnic blood flow undergoes rapid changes. The question has thus arisen whether the BSP method can be applied under these circumstances.

The present investigation is a study of this problem. ESBF was estimated in the normal resting man at constant and at varying BSP plasma concentrations in the same experiment. Consequently, the effect of variations in plasma BSP concentration on flow determinations could be estimated, supposing that the flow was approximately constant during the experiment. However, as the experiments lasted for about one hour, fluctuations in flow values could partly be due to real changes in flow. In order to estimate the importance of the time factor, a separate study was undertaken in which ESBF was repeatedly determined at a constant dye level during two hours.

MATERIAL

Fourteen studies were carried out on twelve healthy, non-hospitalized volunteers without evidence of hepatic disease. Most of them were blood donors. Pertinent data are given in Table I. All had hemoglobin concentrations within the normal range except case No 50, who was anemic.

1 The work described in this paper was aided by grants from the Swedish National Association against Tuberculosis and from the Swedish Institute for Investigations on Malt Beverages.

Table I. *Sex, age and BSA data in the material.*

Case No.	Sex	Age	B.S.A. M^2
44	M	32	1.77
46	F	42	1.62
47	F	36	1.63
51	M	40	1.94
42/48	F	30	1.71
45/40	M	36	1.89
43/35	M	39	2.07
79	M	28	2.07
81	F	21	
39	M	25	1.94
40/45	M	36	1.89
48/42	F	30	1.71
50	M	40	2.00
80	M	29	2.04

METHODS

All experiments were performed in the morning. The subjects, who had fasted since the previous day, were recumbent. Sodium amytal, 0.1 g, was given orally one hour before the start of the experiment. The BSP-solution was injected through a polythene catheter, which was inserted about 10 cm into one of the antecubital veins. Peripheral blood samples were obtained from an indwelling arterial needle or a short polythene catheter placed in one of the brachial arteries.

I. *Determination of the BSP space.*

A single intravenous injection of 100 mg was given at the beginning of the experiment. Arterial samples were obtained after 3, 5, 7 and 9 minutes. The concentration of dye was measured and plotted against time on a semilogarithmic scale. A straight line could always be drawn through these points, thus allowing extrapolation to zero time.

The BSP space was calculated according to the following formula:

$$\text{BSP space, ml} = \frac{\text{injected amount BSP, mg} \times 100}{\text{zero time BSP concentration, mg/100 ml plasma}}$$

II. *Determination of the splanchnic blood flow.*

The method was essentially as described b Bradley *et al.* (1945). The modifications used hav been described in detail elsewhere (Castenfor Eliasch & Hultman 1960).

The right hepatic vein was catheterized using catheter as described by Gidlund (1956) and mod fied by Ödman (1958). The catheter was introduce percutaneously into the femoral vein and, unde fluoroscopic control, passed into the right hepat vein.

A. *Experiments with essentially constant peripher concentration of dye. Fig. 1.*

In four subjects the splanchnic blood flow wa determined repeatedly during two hours. After a intravenous priming dose of 100 mg BSP th sustaining infusion of BSP was administered wit a syringe driven by a motor at a constant rate,

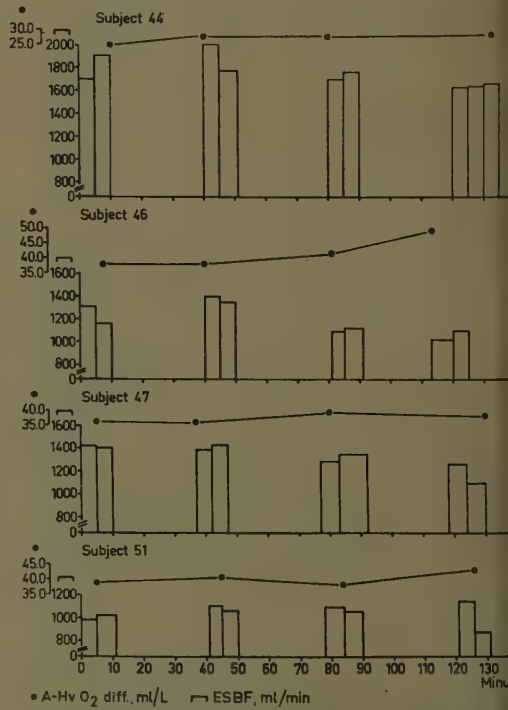
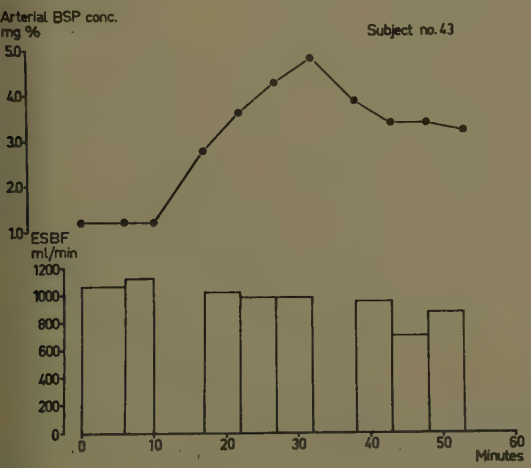


Fig. 1. Experiments with essentially constant perip eral concentration of BSP. 4 experiments.

hat approximately 2 ml of fluid were injected per ninute. The bottle containing the infusion solution was placed on an automatic balance, which allowed ontinous control of the amount of fluid infused. Approximately 3 mg BSP per minute per square neter body surface area were given. After 20—30 ninutes of infusion an equilibrium was obtained be- ween the amounts of dye infused and dye removed, nd sampling was commenced. Three sets of simul- aneous blood samples from the hepatic vein and he brachial artery were drawn at 5-minute inter- als every 30—45 minutes' period.

3. Experiments with continous changes in peripheral concentration of dye.

The infusion rate of BSP was changed twice in ach experiment for a total period of 45 to 60 ninutes. Initially, a priming dose of BPS was iven and the infusion was started as described bove. The first samples were drawn 20—30 mi- utes later. Immediately thereafter, the infusion ate was either increased (5 experiments) or de- reased (5 experiments) and subsequently kept onstant for another period of 20—30 minutes. hen, the infusion rate was changed in the opposite



ig. 2. Experiments with continuous change in the eripheral concentration of BSP; the rise in con- entration was induced before the fall. 1 experiment.

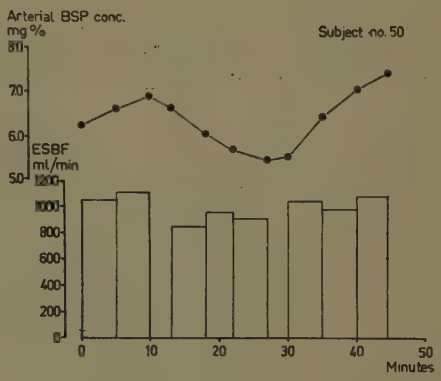


Fig. 3. Experiments with continous change in the peripheral concentration of BSP; the rise in concentration was induced after the fall. 1 experiment.

direction to approximately the initial level and another 20—30 minutes' period of infusion followed. The volumes infused varied between one and four ml per minute. Simultaneous blood samples from the hepatic vein and the brachial artery were drawn throughout the study at approximately 5-minute intervals. The plasma dye concentration patterns thus obtained are illustrated in Figs. 2 and 3.

For technical reasons the infusion of dye was discontinued for one minute before each change in rate. Therefore, there are two short gaps in the estimation of the splanchnic blood flow in each experiment.

METODS OF ANALYSIS

Physiological saline was infused at a slow rate into the catheter in the hepatic vein throughout the experiment. At sampling time the saline solution in the vein catheter was washed out by withdrawing 4 ml of blood which was discarded. Thereafter, samples were taken in heparinized tubes for analysis. The BSP determinations were made in duplicate according to the second method of Gaebler (1945) using the Beckman DU spectrophotometer. The hematocrit was determined by spinning in the

Table II. *Experiments with essentially constan*

Case No.	BSP space ml	Time min	BSP						ESBF			Hematocrit %	Art.-Hep. O_2 diff. ml/L
			Infused amount mg/min	Arterial conc. mg %	Δp mg %/min	Hep. vein conc. mg %	Extraction %	Art.-Hep. vein diff. mg %	ml/min	Mean period 1—2, 3—4, 5—6, 7—8,	Change ml/min		
44	2730	0	5.86	1.21	+ 0.006	0.63	48	0.58	1700	1800		43	
		5		1.24	± 0.000	0.64	48	0.60	1900				27.8
		10		1.24		0.76	39	0.48					
		40	5.86	1.35	+ 0.006	0.90	34	0.46	2000	1890	+ 90	42	28.4
		45		1.38	+ 0.005	0.85	38	0.53	1770				
		51		1,41		0.82	42	0.59					
		80	5.86	1.40	+ 0.002	0.83	41	0.57	1690	1720	— 80	41	
		85		1.41	— 0.008	0.81	43	0.60	1750				27.4
		90		1.37		0.80	42	0.57					
		120	5.81	1.36	+ 0.004	0.78	43	0.58	1620	1630	— 170	41	
		125		1.38	± 0.000	0.76	45	0.62	1630				28.1
		130		1.38		0.79	43	0.59					
46	2750	0	4.72	1.03	— 0.004	0.39	62	0.64	1300	1240		40	
		5		1.01	+ 0.012	0.42	58	0.59	1170				38.0
		10		1.07		0.39	64	0.68					
		40	4.48	0.97	— 0.008	0.42	57	0.55	1390	1370	+ 130	39	37.9
		45		0.93	— 0.002	0.38	59	0.55	1350				
		50		0.92		0.37	60	0.55					
		81	4.62	1.05	— 0.003	0.35	67	0.70	1090	1110	— 130	39	41.4
		85		1.04	— 0.002	0.33	68	0.71	1120				
		91		1.03		0.37	64	0.66					
		113	4.50	1.00	± 0.000	0.27	73	0.73	1020	1060	— 180	38	49.0
		120		1.00	— 0.006	0.31	69	0.69	1090				
		125		0.97		0.29	70	0.68					

Wifug hematocrit centrifuge at a rate of 3 000 r.p.m. for 30 minutes. The oxygen content of the blood was analyzed by the manometric method of van Slyke & Neill (1924). The estimated splanchnic blood flow (ESBF) and BSP extraction ratio were calculated using the following formulae:

$$\text{ESBF, ml/min.} = \frac{\text{Removal} \times 100 \times 100}{(p - h)\ (100 - \text{Hct})}$$

$$\text{Removal} = I \pm \frac{\Delta p \times 0.8 \times \text{BSP space}}{100}$$

$$\text{Extraction ratio} = \frac{(p - h)\ 100}{p}$$

Removal = splanchnic removal of dye, mg/min
I = infusion rate, mg/min
p = arterial = peripheral plasma concentration, mg/100 ml plasma
h = hepatic vein plasma concentration, mg/100 ml plasma
Δp = change in p in mg/100 ml plasma/min
Hct = hematocrit, per cent

The factor 0.8 in the second formula has b used for the following reason: The method is ba on the Fick principle. As the organ on which principle is used is not only the liver but the wh

eripheral concentration of BSP. 4 experiments.

ase No.	BSP space ml	Time min	BSP						ESBF			Hematocrit %	Art.-Hep. O_2 diff. ml/L
			Infused amount mg/min	Arterial conc. mg %	Δ p mg %/min	Hep. vein conc. mg %	Extraction %	Art.-Hep. vein diff. mg %	ml/min	Mean period 1—2, 3—4, 5—6, 7—8,	Change ml/min		
47	2890	0	4.99	1.25		0.64	49	0.61					
		5		1.17	— 0.016	0.57	51	0.60	1430			38	
		10		1.17	± 0.000	0.63	46	0.54	1410	1420			36.3
		37	4.65	1.03		0.51	50	0.52					35.8
		42		1.05	+ 0.004	0.52	50	0.53	1400			38	
		47		1.01	— 0.008	0.46	54	0.55	1440	1420	± 0		
		77	4.85	1.04	+ 0.007	0.45	57	0.59					39.1
		83		1.08		0.46	57	0.62	1290			40	
		92		1.07	— 0.001	0.49	54	0.58	1350	1320	— 100		
		118	4.97	1.15	± 0.000	0.54	53	0.61					
		124		1.15		0.49	57	0.66	1260			38	
		130		1.22	+ 0.012	0.51	58	0.71	1100	1180	— 240		
													38.1
51	3160	0	5.73	1.24		0.28	77	0.96					
		5		1.26	+ 0.004	0.41	67	0.85	990			37	
		11		1.28	+ 0.003	0.38	70	0.90	1020	1010			38.7
		41	5.68	1.52		0.71	53	0.81					
		45		1.52	± 0.000	0.73	52	0.79	1110			36	
		50		1.56	+ 0.008	0.74	53	0.82	1060	1090	+ 80		40.4
		78	5.56	1.81		0.97	46	0.84					
		84		1.90	+ 0.015	1.19	37	0.71	1100			39	
		90		2.02	+ 0.020	1.17	42	0.85	1060	1080	+ 70		38.2
		121	5.45	2.61		1.94	26	0.67					
		126		2.67	+ 0.012	1.87	30	0.80	1150				
		131		2.75	+ 0.016	1.67	39	1.08	880	1020	+ 10		
													43.1

anchnic area, corrections for changes in peri-eral BSP content must be calculated only on the SP space outside this area (extrasplanchnic ace). It is known from earlier work (Bradley, arks, Reynell & Meltzer 1953), that the splanch- plasma volume averages 0.2 x total plasma lume. Hence, 0.8 x BSP space must be used in calculations.

RESULTS

Experiments with essentially constant peripheral concentration of BSP (Table II, and Fig. 1).

In three experiments (Nos. 44, 46 and) the BSP plasma concentration was almost constant during the two hour period. In the fourth (No. 51) there was a slight progressive rise in the BSP plasma concentration and the extraction ratio of BSP fell.

On an average the ESBF fell slightly towards the end of the experiments, but the estimated splanchnic oxygen consumption (product of ESBF and A-HvO_2 difference) was constant.

B. *Experiments with continous change in the peripheral concentration of BSP (Tables III and IV; Figs. 2 and 3).*

The blood flow was measured over a peri-

Table III. *Experiments with continuous change in the peripheral concentrati*

Case No.	BSP space ml	Time min	BSP						ESBF			Hematocrit %	Art.-Hep. O_2 diff.
			Infused amount mg/min	Arterial conc. mg%	Δ p mg %/min	Hep.vein conc. mg %	Extraction %	Art.-Hep. diff. mg %	ml/min	Mean at constant, rising and falling conc.	Change ml/min		
42/48	2720	0.0	4.97	0.83		0.32	61	0.51	1630			36	
		6.0		0.79	— 0.007	0.32	59	0.47	1770	1700			
		11.0		0.76	— 0.006	0.33	57	0.43					
		17.0	8.35	1.05		0.24	77	0.81	1490			37	37
		22.0		1.20	+ 0.030	0.37	69	0.83	1580	1560	— 140		
		27.0		1.26	+ 0.012	0.46	63	0.80	1620				38
		32.0		1.33	+ 0.014	0.55	59	0.78					
		38.0	5.59	1.11		0.47	58	0.64	1550			35	
		44.0		0.97	— 0.023	0.40	59	0.57	1600	1580	— 120		
		49.0		0.96	— 0.002	0.44	54	0.52	1600				33
		54.0		0.94	— 0.004	0.39	59	0.55					33
45/40	3660	0.0	5.28	1.40		0.80	43	0.60	1310			38	42
		5.0		1.46	+ 0.012	0.85	42	0.61	1490	1400			
		10.0		1.50	+ 0.008	1.02	32	0.48					
		16.5	15.15	2.31		1.44	38	0.87	1470			38	43
		21.5		3.31	+ 0.200	2.14	35	1.17	1240	1270	— 130		
		28.5		4.31	+ 0.143	2.63	39	1.68	1090				
		33.5		5.13	+ 0.164	3.74	27	1.39					
		40.0	4.23	4.68		3.63	22	1.05	1090			38	42
		46.0		4.20	— 0.080	3.30	21	0.90	920	1010	— 390		42
		58.0		3.85	— 0.029	2.97	23	0.88					

od of 45 to 60 minutes. The infusion rates ranged between 3.1 and 25.0 mg BSP per minute.

After the initial period of constant infusion the rate was changed. In five experiments (Table III) the infusion rate was first increased and thereafter decreased, resulting in a corresponding change in the peripheral BSP concentration (Fig. 2). In the remaining five (Table IV) the infusion rate was altered in the opposite direction (Fig. 3). In this latter group the initial plasma concentration of BSP was kept at a higher level.

The peripheral BSP concentrations ranged between 0.8 and 7.6 mg BSP per 100 ml plasma; Δ p varied between — 0.206 a + 0.349 mg per cent per minute.

Eight ESBF values were calculated each experiment. The two initial peri with essentially constant peripheral dye c centration were followed by three peri with falling and three periods with ris dye concentration.

The means of the flow values at consta falling and rising concentrations respectiv were calculated for the two types of exp ments; that is, those in which the rise concentration was induced before (Ta III) and those in which it was induced a (Table IV) the fall in concentration.

f BSP, the rise in concentration was induced before the fall. 5 experiments.

Case No.	BSP space ml	Time min	BSP						ESBF			Hematocrit %	Art.-Hep. O_2 diff. ml/L
			Infused amount mg/min	Arterial conc. mg %	Δp mg %/min	Hep. vein conc. mg %	Extraction %	Art.-Hep. diff. mg %	ml/min	Mean at constant, rising and falling conc.	Change ml/min		
-3/35	4190	0.0	5.89	1.21	± 0.000	0.27	78	0.94	1070	1100		42	54.7
		6.0		1.21	— 0.003	0.26	79	0.95	1130				
		10.0		1.20		0.33	73	0.87					
		17.0	19.20	2.75	+ 0.168	0.69	75	2.06	1030			42	57.0
		22.0		3.59	+ 0.130	1.11	69	2.48	990	1000	— 100		
		27.0		4.24	+ 0.102	1.56	63	2.68	990				55.9
		32.0		4.75		1.93	59	2.82					
		38.0	6.14	3.82	— 0.098	1.88	51	1.94	960				
		43.0		3.33	± 0.000	1.81	46	1.52	710	850	— 250	43	50.7
		48.0		3.33	— 0.038	1.82	45	1.51	880				
		53.0		3.14		1.71	46	1.43					
79	3140	0.0	7.18	1.84	— 0.022	0.29	84	1.55	910	900		42	
		5.5		1.72	± 0.000	0.33	81	1.39	890				57.5
		11.0		1.72		0.33	81	1.39					
		16.0	25.00	3.30	+ 0.325	0.50	85	2.80	950			44	
		20.0		4.60	+ 0.193	1.05	77	3.55	940	920	+ 20		
		25.5		5.66	+ 0.172	1.53	73	4.13	860				58.1
		30.5		6.52		2.10	68	4.42					
		36.0	11.07	5.69	— 0.116	2.59	54	3.10	870			44	
		41.0		5.11	— 0.022	2.44	52	2.67	820	870	— 30		58.8
		46.0		5.00	— 0.036	2.59	48	2.41	920				60.2
		51.0		4.82		2.58	46	2.24					
81	2090	0.0	5.34	1.46	— 0.004	0.68	53	0.78	1230	1240		37	41.7
		5.5		1.44	+ 0.016	0.81	44	0.63	1250				
		10.0		1.51		0.85	44	0.66					
		15.5	17.67	3.00	+ 0.185	1.71	43	1.29	1570			37	
		21.0		4.02	+ 0.189	2.36	41	1.66	1210	1360	+ 120		39.4
		25.5		4.87	+ 0.134	2.73	44	2.14	1300				
		30.5		5.54		3.91	29	1.63	1250			36	
		38.0	5.67	4.45	— 0.160	3.28	26	1.17					40.9
		40.5		4.05	— 0.064	3.13	23	0.92	1230	1210	— 30		36.4
		45.5		3.73	— 0.024	2.94	21	0.79	1150				
		50.0		3.62		2.76	24	0.86					

ıean flow at constant concentration was ubtracted from the means at rising and alling concentrations, respectively. The ıeans of the obtained differences are listed ı Table V.

The calculated mean flow at a falling concentration of BSP was lower irrespective of whether the fall in concentration was in the earlier or the later part of the experiment. This was significant ($0.01 > P > 0.001$) in the Table IV type of experiment and not significant ($0.1 > P > 0.05$) in the Table III type of experiment.

No correlation was found between the

Table IV. *Experiments with continuous change in the peripheral concentrati*

Case No.	BSP space ml	Time min	BSP					ESBF				Hematocrit %	Art.-Hep. O_2 diff. ml/l
			Infused amount mg/min	Arterial conc. mg %	Δ p mg %/min	Hep. vein conc. mg %	Extraction %	Art.-Hep. diff. mg %	ml/min	Mean at constant, falling and rising conc.	Change ml/min		
39	2930	0.0	7.11	1.12		0.38	67	0.74	1600			38	
		6.0		1.12	± 0.000	0.43	61	0.69	1540	1570			
		11.0		1.19	+ 0.014	0.46	61	0.73					
		20.0	4.69	0.89		0.36	59	0.53	1610			40	
		25.0		0.82	— 0.014	0.31	62	0.51	1460	1510	— 60		
		30.0		0.82	± 0.000	0.26	68	0.56	1450				
		35.0		0.83	+ 0.002	0.32	62	0.51					
		43.0	7.17	1.08		0.48	56	0.60	1770			39	
		48.0		1.15	+ 0.014	0.48	58	0.67	1670	1700	+ 130		
		57.0		1.24	+ 0.010	0.55	55	0.69	1650				
		62.0		1.28	+ 0.008	0.58	55	0.70					
40/45	2660	0.0	7.22	2.82		2.02	28	0.80	1230			40	
		5.0		2.93	+ 0.022	1.93	34	1.00	1150	1190			
		11.0		3.10	+ 0.028	2.20	29	0.90					
		17.0	3.09	2.73		2.08	24	0.65	1090			39	
		22.0		2.52	— 0.042	1.92	24	0.60	1060	1100	— 90		
		27.0		2.38	— 0.028	1.81	24	0.57	1140				
		32.0		2.26	— 0.024	1.77	22	0.49					
		39.0	5.45	2.37		1.78	25	0.59	1380				
		44.0		2.45	+ 0.016	1.82	26	0.63	1320	1300	+ 110	40	
		49.0		2.54	+ 0.018	1.91	25	0.63	1210				
		54.0		2.63	+ 0.018	1.89	28	0.74					
48/42	2660	0.0	16.31	4.16		1.99	52	2.17	1020			35	39.
		5.0		4.50	+ 0.068	2.17	52	2.33	1060	1040			
		9.0		4.61	+ 0.028	2.38	48	2.23					
		13.0	7.92	4.16		2.33	44	1.83					
		17.5		3.74	— 0.093	2.05	45	1.69	880			36	
		22.5		3.29	— 0.090	1.72	48	1.57	940	910	— 130		39.
		27.0		3.13	— 0.036	1.76	44	1.37	920				
		30.0	16.76	3.14		1.64	48	1.50					
		35.0		4.08	+ 0.188	1.99	51	2.09	1090			35	
		39.0		4.57	+ 0.123	2.49	46	2.08	1040	1100	+ 60		33.
		44.0		4.75	+ 0.036	2.67	44	2.08	1180				33.

magnitude of Δ p and the order of apparent change in blood flow. Furthermore, there was no relationship between the order of initial flow values and the apparent change in flow.

In eight of the ten experiments the arteriohepatic venous oxygen difference was determined four times in each. The oxygen di ference varied only slightly in the majori of the experiments and there was no dire tional change with time.

DISCUSSION

In a previous investigation (Castenfors *al.* 1960) it was found that variations in t

BSP; the rise in concentration was induced after the fall[1]. 5 experiments.

ase o.	BSP space ml	Time min	BSP					ESBF				Hematocrit %	Art.-Hep. O_2 diff. ml/L
			Infused amount mg/min	Arterial conc. mg %	Δ p mg %/min	Hep. vein conc. mg %	Extraction %	Art.-Hep. diff. mg %	ml/min	Mean at constant, falling and rising conc.	Change ml/min		
0	3040	0.0	16.98	6.26		3.97	37	2.29					
		5.0		6.62	+ 0.072	4.70	29	1.92	1050	1080		31	82.2
		10.0		6.92	+ 0.060	4.80	31	2.12	1110				
		13.0	6.99	6.67		4.84	27	1.83					
		18.0		6.08	— 0.118	4.50	26	1.58	850			32	91.2
		22.0		5.75	— 0.083	4.58	20	1.17	960	910	— 170		
		27.0		5.49	— 0.052	4.00	27	1.49	910				
		30.0	17.53	5.58		3.91	30	1.67					
		35.0		6.45	+ 0.174	4.41	32	2.04	1040			31	80.8
		40.0		7.11	+ 0.132	4.91	32	2.20	980	1030	— 50		
		44.5		7.48	+ 0.082	5.49	71	1.99	1080				89.5
0	3530	0.0	16.11	7.38		5.47	26	1.91					
		5.0		7.50	+ 0.024	5.77	23	1.73	1440	1450		41	46.8
		10.0		7.57	+ 0.014	5.66	25	1.91	1460				
		15.0	4.15	6.54		5.09	22	1.45					
		20.0	3.96	5.51	— 0.206	4.31	22	1.20	1280	1310	— 140	41	56.1
		30.0		3.81	— 0.170	2.79	27	1.02	1340				
		35.0	20.69	5.01		3.24	35	1.77					
		40.5		6.10	+ 0.198	3.86	37	2.24	1280			41	51.6
		45.0		6.56	+ 0.102	4.39	33	2.17	1370	1350	— 100		
		50.0		7.04	+ 0.096	4.89	31	2.15	1410				52.4

Table V. *The deviation in calculated splanchnic blood flow during variation of the peripheral plasma concentration of BSP.*

Plasma concentration	Mean flow at constant concentration	Mean flow at rising conc. — mean flow at constant conc.	Mean flow at falling conc. — mean flow at constant conc.
Rise preceding fall	1268 ml/min	—46 ml/min (—3.6%)	—164±71 ml/min (—12.9%)
Fall preceding rise	1266 ml/min	+32 ml/min (+2.5%)	—119±19 ml/min (— 9.4%)

[1] Case No. 50: Remarkably high A-HvO_2 difference and also the lowest oxygen content of the erial blood (oxygen capacity 139 ml/L).

sma concentration of BSP ranging be- een 0.5 and 10 mg per 100 ml plasma had influence on the result of determination of splanchnic blood flow, provided that the concentration was kept constant during each separate period of estimation.

In clinical and physiological studies in man, marked continous variations in peri-

pheral BSP concentration sometimes occur when the splanchnic blood flow varies. This is mainly due to changes in the hepatic clearance of dye. Therefore, it was also considered important to study the validity of the method when the peripheral BSP concentration was subject to continous change. In order to correct for the changes in the peripheral plasma dye content, the change in peripheral dye content per minute ($\Delta p \times 0.8 \times$ BSP space) was either added to or subtracted from the amount of BSP infused per minute.

While the determination of Δp involves only colorimetric errors, the estimation of the BSP space may be subject to criticism. According to Ingelfinger, Bradley, Mendeloff & Kramer (1948) the BSP space is approximately equal to the plasma volume. The spaces of two similar dyes, indocyanine green (Cherrick, Stein, Leevy & Davidson 1960) and Rose Bengal (Sapirstein & Simpson 1955), have also been found to be equal to the plasma volume. However, most investigators have employed Evans' blue dye,

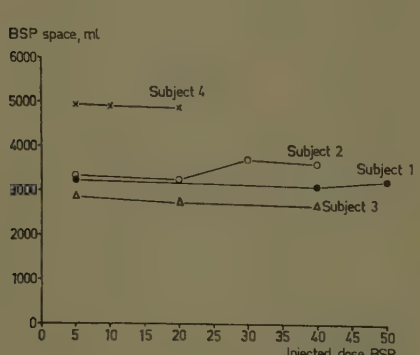


Fig. 4. The BSP space determined in the same individual by giving different amounts of BSP as single intravenous injection. 4 subjects.

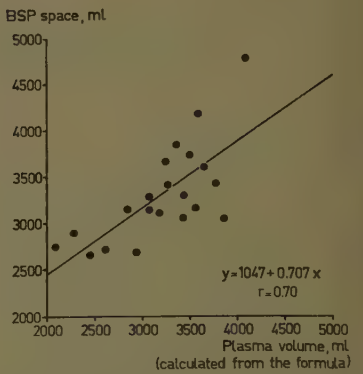


Fig. 5. The relationship between the BSP space the plasma volume calculated according to strand (1949).

and more recently, I^{131} albumin to determ the plasma volume, when estimating splanchnic or hepatic blood flow with BSP method. Others (Sapirstein *et al.* 19 claim that the BSP space is larger than plasma volume. In this study the BSP sp was measured directly since the other pr dures were considered impracticable for t nical reasons. Furthermore, in this inve gation, it would be more correct to meas the volume of distribution of BSP ra than that of any other substance, even if errors inherent in the procedure of ex polation to zero time are greater using B which is more rapidly excreted than Ev blue dye and I^{131} albumin. In addition, validity of the present procedure is suppo by earlier findings, namely that the B space is independent of the dose administ (Hultman & Castenfors 1957), as exem fied in Fig. 4. The calculated BSP sp correlated well to the plasma volume as dicted according to the formula of Sjöst (1949) (Fig. 5).

Since the aim of this investigation wa

udy whether induced variations in the eripheral plasma concentration of BSP ould lead to calculated splanchnic blood ow values outside the range of physiological ariations in the resting individual, it was ecessary to perform control studies in which constant dye level was obtained. Similar udies have been made by Sherlock, Bearn, illing & Paterson (1950), who determined e splanchnic blood flow for several hours a constant BSP concentration. No system-ic change in blood flow was observed when e dye level was kept above 1 mg BSP per 00 ml plasma. In the present investigation, ing the same procedure, the calculated ood flow on an average decreased only ightly towards the end of a two-hour eriod.

In the ten experiments in which large anges in the peripheral dye concentration ere induced, the calculated splanchnic ood flow values varied only slightly in most periments. Only in two did the calculated ow values deviate as much as 35 per cent om the mean flow, estimated during the o first periods with constant peripheral e concentration. The flow calculated at creasing peripheral BSP concentration as slightly lower[1]) than the mean of the o first periods with constant peripheral ncentration, irrespective of whether the crease in concentration was induced dur-g the earlier or the later part of the study. us, the lower flow values at decreasing e concentration cannot be explained by ontaneous changes in blood flow. There as a tendency towards a decrease in blood flow at the end of the period in three out of four experiments, in which ESBF was estimated with the conventional technique. However, in these experiments blood flow was estimated for about two hours instead of 45—70 minutes as in the experiments with induced variations in the peripheral BSP concentration. Hence, the lower flow values at decreasing concentration are likely to be due to systematic errors in methods. The explanation could be:

1. that the BSP space is underestimated. Such an error should render lower flow values at decreasing concentration but also higher values at increasing concentration. However, flow values at rising concentration were not higher than those obtained at constant concentration and hence, an underestimation of the BSP space cannot explain the results.

2. that extrahepatic uptake and release of BSP influenced the flow measurement. It has long been known that a small part of injected BSP is taken up by tissues other than the liver parenchyma (Brauer, Pessotti & Krebs 1955, Selkurt 1953 and 1954, and others). Extrahepatic uptake of BSP should theoretically depend on the concentration of dye in the blood. This means that at constant (Brauer *et al.* 1955) as well as at rising blood concentrations BSP is taken up by extrahepatic tissue, and that at falling dye concentrations BSP is released from extrahepatic tissue. This should result in falsely high flow values at constant and rising dye concentration and in falsely low flow values at falling dye concentration. Thus, the existence of a small extrahepatic uptake and re-

[1] The subject with anemia did not differ from others.

lease of BSP may explain the small differences between flow values obtained at constant and falling dye concentration in the majority of the experiments.

However, it was recently (Wheeler, Epstein, Robinson & Snell 1960) demonstrated by direct measurement in animal experiments that the extrahepatic uptake is small at normal hepatic function when the BSP plasma level is constant. These findings were in agreement with earlier studies in man (Castenfors *et al.* 1960). According to these findings, it is impossible to explain the great differences in flow values obtained in some of the present experiments. It is suggested that these discrepancies may be explained by individual differences in BSP storage and excretion capacity and by release of BSP from the liver at falling dye concentration. The BSP liver removal consists of two separate processes: one is storage of the dye by the liver cells and the other is excretion of the dye into the bile (Wirts & Cantarow 1942, Brauer & Pessotti 1950, Wheeler *et al.* 1960, Wheeler, Meltzer & Bradley 1960, Hultman *et al.* 1957, and others). There is strong evidence for the existence of a concentration gradient between the liver cells and plasma (Wheeler, Meltzer & Bradley 1960). Consequently BSP stored in the liver cells will rediffuse into the blood when the dye concentration in blood decreases. T error in flow determination due to extr hepatic uptake and release of BSP is t same as the error in the calculated remov The relative magnitude of this error can expressed by the following quotients.*

Hence, the error will increase when t hepatic excretion of dye is small. The err at rising concentration may be equal to t error at constant concentration; the larg extrahepatic uptake at rising concentrati is balanced by the hepatic storage of dy The error at falling concentration can expected to be more marked in cases whe the hepatic excretion of BSP into the b is small and the release of BSP from t liver cells is large. This could be suspect in experiments Nos. 43 and 45.

Bradley (1956) states that the ba splanchnic blood flow should be determin at arterial plasma concentration where Δ does not exceed a value of 0.005 mg per 1 ml plasma per minute. According to the p sent results there are small variations in c culated flow values in spite of large Δ (range — 0.206 + 0.349 mg per 100 plasma per minute). An exception is countered when the plasma BSP conc tration is rapidly falling. In this case variation in flow values may reach 35 cent. It should be pointed out that the

* At constant concentration: $+\frac{\text{extrahepatic uptake}}{\text{hepatic excretion}}$

At rising concentration: $+\frac{\text{extrahepatic uptake}}{\text{hepatic excretion} + \text{hepatic storage}}$

At falling concentration: $-\frac{\text{extrahepatic release}}{\text{hepatic excretion} - \text{hepatic release}}$

ced variations in dye concentration in the esent investigation were of a much higher der than those observed when the splanch- c blood flow increased markedly after ally administered glucose (Castenfors, iasch & Hultman 1960).

In the present investigation only subjects th normal hepatic function were studied. ne systematic errors would probably be eater if the same study had been under- ken on patients with liver disease. This ust be the reason for dubious flow values metimes encountered in studies of the er blood flow in cases with hepatic cirrho- s.

SUMMARY

1. The splanchnic blood flow was esti- ated with the bromsulphalein (BSP) ethod on healthy, non-hospitalized volun- ers.

2. When BSP was infused at a constant te for a two hour period, the estimated lanchnic blood flow decreased with time in ree out of four experiments.

3. When BSP was infused at varying tes in the same experiment, resulting in ntinous increase or decrease in the BSP asma concentration, the calculated splanch- c blood flow was found to be lower at a lling plasma concentration of BSP as com- red to values obtained at constant plasma ncentration of BSP.

4. It is suggested that the lower values in lculated splanchnic blood flow obtained at falling BSP concentration are due to a lease of extrahepatically stored BSP into e plasma compartment.

ACKNOWLEDGMENT

The authors wish to thank Miss Geneviève Leballeur, Miss Gill Robertson, Mrs Margareta Söderling-Furu and Mrs Anna-Brita Ohlson for technical assistance. We are indebted to the hospital pharmacist R. Gartner for the preparation of all infusion solutions.

REFERENCES

Bradley, S. E.: Die Messung der Leberdurchblutung von Mensch und Tier. Klin. Wchnschr. *34*, 44, 1956.

Bradley, S. E., Ingelfinger, F. J., Bradley, G. P. & Curry, J. J.: Estimation of hepatic blood flow in man. J. Clin. Investigation *24*, 890, 1945.

— Marks, P. A., Reynell, P. S. & Meltzer, J.: The circulating splanchnic blood volume in dog and man. Tr. A. Am. Physicians *66*, 294, 1953.

Brauer, R. W. & Pessotti, R. L.: Hepatic uptake and biliary excretion of bromsulphthalein in the dog. Am. J. Physiol. *162*, 565, 1950.

— Pessotti, R. L. & Krebs, J. S.: The distribution and excretion of S^{35}-labeled sulfo-bromophtalein-sodium administered to dogs by continuous infusion. J. Clin. Investigation *34*, 35, 1955.

Castenfors, H., Eliasch, H. & Hultman, E.: The splanchnic blood flow and oxygen consumption estimated in man by the bromsulphalein method with special reference to the influence of the peripheral dy level. Scandinav. J. Clin. & Lab. Investigation *12*, 158, 1960.

— Eliasch, H. & Hultman, E.: Dumpingsyndromets patofysiologi. Nord. med. *63*, 637, 1960.

Cherrick, G. R., Stein, S. W., Leevy, C. M. & Davidson, C. S.: Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. J. Clin. Investigation *39*, 592, 1960.

Gaebler, O. H.: The determination of bromsulphalein in normal, turbid, hemolyzed, or icteric serum. Am. J. Clin. Path. *15*, 452, 1945.

Gidlund, Å.: Development of apparatus and methods for roentgen studies in haemodynamics. Acta radiol. Suppl. 130, 1956.

Hultman, E. & Castenfors, H.: Some aspects of the bromsulphthalein disappearance curve on giving single high doses. Scandinav. J. Clin. & Lab. Investigation *10,* Suppl. 31, 278, 1957 & International Meeting of clin. chem., Stockholm, 1957.

Ingelfinger, F. J., Bradley, S. E., Mendeloff, A. I. & Kramer, P.: Studies with bromsulphalein. I. Its disappearance from the blood after a single intravenous injection. Gastroenterology *11,* 646, 1948.

Ödman, P.: Percutaneous selective angiography of the coelic artery. Acta radiol. Scandinav. Suppl. 159, 1958.

Sapirstein, L. A. & Simpson, A. M.: Plasma clearance of Rose Bengal (Tetraiodotetrabromfluorescein). Am. J. Physiol, 182, 337, 1955.

Selkurt, E. E.: Validity of the bromsulphalein (BSP) method for estimating hepatic blood flow. Am. J. Physiol. *175,* 461, 1953.

— Comparison of the bromsulphalein method with simultaneous direct hepatic blood flow. Circulation Research *2,* 155, 1954.

Sherlock, S., Bearn, B. A., Billing, B. H. & Paterson, J. C. S.: Splanchnic blood flow in man by the bromsulphalein method; the relation peripheral plasma bromsulphalein level to calculated flow. J. Lab. & Clin. Med. *35,* 9 1950.

Sjöstrand, T.: The total quantity of hemoglobin man and its relation to age, sex, bodyweig and height. Acta physiol. Scandinav. *18,* 3 1949.

van Slyke, D. D. & Neill, J. M.: The determinati of gases in blood and other solutions by vac um extraction and manometric measuremen VII. The determination of oxygen in blood. Biol. Chem. *61,* 552, 1924.

Wheeler, H. O., Epstein, R. M. Robinson, R. R. Snell, E. S.: Hepatic storage and excretion sulfobromophthalein sodium in the dog. Clin. Investigation *39,* 236, 1960.

— Meltzer, J. I. & Bradley, S. E.: Biliary tra port and hepatic storage of sulfobromophthal sodium in the unanesthetized dog, in norm man, and in patients with hepatic disease. Clin. Investigation *39,* 1131, 1960.

Wirts, C. W. Jr. & Cantarow, A.: A study of t excretion of bromsulphthalein in the bile. A J. Dig. Dis. *9,* 101, 1942.

candinav. J. Clin. & Lab. Investigation 503—511, 13, 1961.

EFFECT OF INJECTION OF HYPERTONIC GLUCOSE ON PLANCHNIC BLOOD FLOW AND OXYGEN CONSUMPTION[1]

By E. HULTMAN and H. CASTENFORS

From the Central Laboratory and the 2nd Medical Service, S:t Eriks Hospital, Stockholm, Sweden.

(Received for publication February 6, 1961)

By use of modern vein catheterization chnique the hepatic circulation and meta-olism have recently been extensively exam-ed and results have been reported by sev-al investigators. Nevertheless our know-dge of the influence of glucose on these nctions is limited. As glucose is central in ver physiology and has a vast use in thera-, more knowledge on this subject was sumed to be of value.

The effect of intravenous glucose admini-ration on the splanchnic circulation and xygen metabolism in man has been studied Myers (1948). He stated that glucose ven intravenously diminished the splanch-c blood flow as well as splanchnic oxygen nsumption.

In this paper new data on this subject will presented. The results obtained indicate at injections of hypertonic glucose solu-ons do not influence splanchnic blood flow splanchnic oxygen metabolism.

MATERIAL AND METHODS

Six healthy non-hospitalized individuals were studied. The splanchnic blood flow (ESBF) was estimated by use of bromsulphalein (BSP) according to Bradley, Ingelfinger, Bradley & Curry (1945). All experiments were performed in the morning. The subjects were recumbent and had fasted since the foregoing night. No premedication was given. The right hepatic vein was catheterized after percutaneous introduction of the catheter into the right femoral vein (Castenfors, Eliasch & Hultman 1960). Two small polythene catheters were then introduced, one in one of the brachial arteries and the other in a cubital vein. First the BSP space was determined by giving a single injection of 100 mg BSP in the cubital catheter (Castenfors *et al.* 1960). Immediately thereafter a priming dose of 100 mg BSP was administrated and the cubital vein catheter was connected to the BSP infusion pump. BSP was given at an approximate rate of 3 mg per minute per square meter body surface area throughout the experiment. After 30 minutes of infusion, sampling was commenced. Blood was drawn simultaneously from the right hepatic vein and the brachial artery at 5 to 10 minute intervals throughout the experiment. After two initial periods to determine the basal data, a 30 per cent glucose solution was given in another cubital vein during 3 to 14 minutes. In each experiment 50 g of glucose was administered. The effect of the glucose injection was studied during 40 to

[1] The work described in this paper was aided by ants from the Swedish National Association gainst Tuberculosis and from the Swedish Insti-e for Investigations on Malt Beverages.

55 minutes. Samples were analyzed for bromsulphalein, hematocrit, hemoglobin and oxygen as described earlier (Castenfors *et al.* 1960). The blood glucose content was measured in duplicate using the orthotoluidine method (Ek & Hultman 1958 and Hultman 1959). The estimated splanchn blood flow (ESBF), the splanchnic oxygen co sumption (SOC), the splanchnic glucose productic (SGP) and the BSP extraction ratio were ca culated from the following formulae:

$$\text{ESBF ml/min} = \frac{100 \times \text{Removal}}{(p - h) \times \frac{100 - \text{Hct}}{100}}$$

$$\text{Removal mg/min} = I \pm \frac{\Delta p}{100} \times 0.8 \times \text{BSP space}$$

$$\text{SOC ml/min} = 0.001 \times \text{ESBF} \times \text{A-Hv}O_2 \text{ diff.}$$

$$\text{SGP mg/min} = 0.01 \times \text{ESBF} \times \text{Hv-A glucose diff.}$$

Removal = amount BSP removed by the splanchnic area (mg/min)

ESBF = estimated splanchnic blood flow (ml/min)

I = infusion rate (mg/min)

p = arterial plasma concentration of BSP (mg/100 ml plasma)

h = hepatic vein plasma concentration of BSP (mg/100 ml plasma)

Δp = change in p in mg/100 ml plasma per min

Hct = hematocrit (per cent)

A-HvO_2 diff. = difference in oxygen content between arterial and hepatic vein blood (ml/l

Hv-A glucose diff. = difference in glucose content between hepatic vein and arterial blood (mg/1 ml blood)

BSP space (ml) was calculated from:

$$\frac{\text{injected amount BSP mg/min}}{0.01 \times \text{zeroconc. BSP/100 ml plasma}}$$

As the injection of hypertonic glucose caused an expansion of the plasma volume or the BSP space, the removal could not be calculated from the usual formula ($I \pm \frac{\Delta p}{100} \times 0.8 \times$ BSP space). Instead the following formula was used to correct for t change in the extrasplanchnic BSP amount duri an alteration of the plasma volume (BSP space

$$\text{Removal} = I \pm \frac{0.8\ (\text{BSP space}_1 \times p_1 - \text{BSP space}_2 \times p_2)}{100 \times t}$$

The correctness of this formula necessitates a uniform change in the extrasplanchnic and the splanchnic plasma volumes.

The BSP space, when changed, was calculated from the hematocrit and the hemoglobin valu according to Elkinton & Danowski (1956). T formula is valid when the total hemoglobin amou does not change.

$$\text{BSP space}_2 = \text{BSP space}_1 \times \frac{Hb_1\ (100 - Hct_2)}{Hb_2\ (100 - Hct_1)}$$

t = period time, minutes ($t_2 - t_1$)

BSP space$_1$, ml = BSP space at time$_1$

BSP space$_2$, ml = BSP space at time$_2$

p_1, mg/100 ml plasma = BSP concentration at time$_1$

p_2, mg/100 ml plasma = BSP concentration at time$_2$

RESULTS (Table I, Fig. 1).

The mean ESBF before the glucose injection was 1450 ml per minute and the mean ESBF after glucose was 1490 ml per minute. The corresponding values for the splanchnic oxygen consumption were 53.3 and 54.5 ml per minute.

The mean splanchnic glucose production the two initial periods was calculated as 9 mg per minute (range 120—490). The maximal blood glucose concentration, appearing immediately after the glucose injection, ranged between 310 and 420 mg per 00 ml blood (mean 360). The blood glucose concentration then decreased continuously to mean value of 175 mg pr. 100 ml blood range 150—220) at 45 minutes. In five out six experiments the glucose maximum appeared in the hepatic vein. In the sixth (No. ?) maximum appeared in the artery. This se had the largest basal splanchnic glucose production (490 mg/min).

After the peak in blood glucose concentration the difference between hepatic vein and artery glucose concentrations was small negative. In three out of six experiments e blood glucose content of the inferior caval in below the entrance of the renal veins as also determined. In two of these experiments (Nos. 108 and 112) the caval glucose concentration was lower than the arterial concentration both during the initial periods d after the glucose injection. In experiment No. 100 no such difference was observed.

In one experiment (No. 82) the hemoglobin content of the heaptic vein blood was greater than that of the arterial blood during e two initial periods. In experiment No. 2 the reverse relationship was found. In all experiments there was a diminution of the hemoglobin concentration of the blood after the glucose injection. The maximal changes were observed in the blood samples drawn immediately after the glucose injection. The average increase in the initially determined BSP space amounted to + 156 ml.

DISCUSSION

It has earlier been reported that a glucose solution given either intravenously (Myers 1948) or orally (Brandt, Castleman, Ruskin, Greenwald & Kelly 1955) decreases the splanchnic blood flow as well as the splanchnic oxygen consumption. In the present investigation no effect of intravenous injections of a hypertonic glucose solution was observed on either the splanchnic blood flow or the splanchnic oxygen consumption. Myers' data (1948) were presented as a short summary and no details were given. According to our findings glucose administrated by different routes has different effects on the splanchnic circulation. In a previous investigation (Castenfors *et al.* 1960) it was found, contradictory to Brandt *et al.* (1955) that hypertonic glucose given orally markedly increased the splanchnic blood flow. Thus, it was found important to examine whether the increase of the splanchnic blood flow was due to the hyperglycemia obtained in these studies.

In the present study it was necessary to correct for changes of the BSP space when calculating the blood flow: the diminution of the plasma BSP concentration (negative Δp) immediately after the glucose injection was not due to an increased removal of dye but to the rapid expansion of the BSP space or

Table I. *The effect of intravenous injection of 50 gm glucose studied on s (ESBF), splanchnic oxygen consumptio*

Subj. No.	Sex	Age	BSA m²	BSP space ml	Time from start of glucose inj. min	Period time min	BSP			
							Infused amount mg/min	Arterial plasma conc. mg %	Δ p mg %/min	Extraction %
82	M	26	2.07	2912	— 12.5	5.3	6.77	1.33	+ 0.011	79
					— 7.5	5.0	6.71	1.39	+ 0.006	71
					— 2.5			1.42		68
	50 gm glucose i. v. (0—3 min)			3081		6.1	6.64		— 0.028	
				3161	3.5	5.9	6.58	1.25	+ 0.003	71
					9.5	4.5	6.44	1.27	+ 0.020	69
					14.0	4.7	6.83	1.36	+ 0.026	69
					18.5	4.8	6.58	1.48	+ 0.010	74
					23.5	5.8	6.55	1.53	+ 0.014	69
					29.0	9.5	6.70	1.61	+ 0.012	73
				3101	39.0			17.2		67
99	M	24	2.15	3649	— 11.0	5.1	6.54	1.27	+ 0.006	61
					— 6.0	4.8	6.56	1.30	+ 0.004	62
					— 1.5			1.32		59
	50 gm glucose i. v. (0—4.5 min)			4113		9.0	6.73		— 0.026	
					7.5	5.6	6.71	1.09	+ 0.018	61
					13.5	5.3	6.51	1.19	+ 0.015	51
					18.5	4.9	6.63	1.27	— 0.016	54
					23.5	5.5	6.62	1.19	± 0.000	53
					29.0	5.0	6.53	1.19	+ 0.024	52
					34.0	10.0	6.53	1.31	— 0.008	47
					44.0			1.23		52
100	M	31	2.04	3205	— 13.0	5.2	6.72	1.03	+ 0.002	63
					— 7.5	5.3	6.56	1.04	+ 0.004	58
					— 2.5			1.06		57
	50 gm glucose i. v. (0—7 min)			3425		11.7	6.37		— 0.009	
				3523	9.5	5.3	6.21	0.95	+ 0.017	55
					14.5	4.7	6.29	1.04	+ 0.011	57
					19.5	5.3	6.47	1.09	+ 0.017	47
					24.5	4.9	6.45	1.18	+ 0.010	46
					29.5	5.1	6.49	1.23	+ 0.012	46
					34.5	10.7	6.39	1.29	— 0.001	45
					45.5			1.28		52

the plasma volume. Instead of directly measuring the change of the plasma volume, hematocrit and hemoglobin were determined on each blood sample and the changes of the plasma volume were calculated from the hematocrit and hemoglobin values according to the formula given in "methods". However, the alterations of the BSP space, thus countered, were small and had no influe on the determination of the flow except the first period after the glucose injection.

In the results values are given of splanchnic glucose production (calculated the product of flow and Hv — A gluc

ormal volunteers. Values are given for estimated splanchnic blood flow nd splanchnic glucose production.

ESBF ml/min	A - Hv O_2 diff. ml/L	Splanchnic O_2 consumption ml/min	Hematocrit %	Hemoglobin extinction		Blood glucose mg %			Hv - A glucose diff. mg %	Glucose production mg/min
				Artery	Hep. vein	Artery	Hep. vein	Inferior caval vein		
1060	—	—	39.5	.537	.563	86	135		49	
1110	31.7	34.4	39.0	.543	.561	86	130		44	492.9
1240	—	—	39.5	.547	.571	86	130		44	488.4
1180	31.6	38.2	38.5	.495	.549	368	318		— 50	— 37.2
0160	28.0	31.4	38.5	.513	.535	270	303		33	— 100.3
980	—	—	38.5	.521	.536	239	246		7	212.0
950	—	—	—	.516	.555	222	232		10	83.3
900	30.5	28.2	38.0	.522	.547	218	196		— 22	— 57.0
900	—	—	38.0	.515	.546	200	170		— 30	— 234.0
	—	—	37.5	.538	.562	174	170		— 4	— 153.0
1440	—	—	44.0	.622	.627	78	97		19	
1470	40.0	58.2	—	.616	.629	74	98		24	309.6
1710	—	—	45.0	.619	.618	77	95		18	308.7
1650	39.6	66.5	42.0	.579	.578	310	331		21	333.5
1600	34.8	56.6	42.0	.585	.588	250	254		4	206.3
1880	—	—	42.0	.587	.596	229	230		1	40.0
1860	—	—	43.0	.598	.595	208	210		2	28.2
1610	39.1	67.8	43.0	.592	.588	188	189		1	27.9
1880	—	—	42.0	.603	.584	173	149		— 24	— 185.2
	—	—	43.0	.606	.598	154	152		— 2	— 244.4
1660	—	—	—	.519	.513	79	95	81	16	
1660	45.2	75.0	35.5	.521	.505	79	94	81	15	257.3
1780	—	—	35.0	.518	.507	75	84	76	9	199.2
1550	43.6	72.6	35.5	.468	.471	329	346	332	17	231.4
1680	47.3	76.4	34.5	.486	.465	291	290	292	— 1	124.0
1770	—	—	36.0	.487	.480	261	261	260	± 0	8.4
1740	—	—	35.0	.500	.490	243	247	246	4	35.4
1680	48.2	82.4	—	.490	.499	206	220	241	14	156.6
1640	—	—	37.0	.489	.493	198	206	221	— 4	184.8
	—	—	37.0	.504	.486	185	181	193	8	— 32.8

lifference). These data are uncertain in subjects with a rapidly falling blood glucose concentration as after a single intravenous injection of hypertonic glucose. The blood samples from the artery and the hepatic vein were drawn simultaneously and thus, at a rapidly falling blood concentration, the hepatic vein value should be higher than the arterial value because of the circulatory delay over the splanchnic vascular system. Furthermore, at a falling concentration the splanchnic glucose values must be influenced by rapid uptake and release of glucose from different tissues. For this reason the splanch-

Table I cont. *The effect of intravenous injection of 50 gm glucose studied on si (ESBF), splanchnic oxygen consumptio*

Subj. No.	Sex	Age	BSA m²	BSP space ml	Time from start of glucose inj. min	Period time min	BSP			
							Infused amount mg/min	Arterial plasma conc. mg %	Δ p mg %/min	Extraction %
101	M	24	1.91	2999	— 12.0	5.5	6.35	0.89	— 0.002	57
					— 6.5	5.1	6.54	0.88	+ 0.012	57
					— 1.5	10.6	6.25	0.94	— 0.009	56
	50 gm glucose i. v. (0—7 min)			3306	9.0	5.2	5.97	0.84	+ 0.013	55
					14.0	4.9	6.14	0.91	+ 0.014	49
					19.0	4.9	6.21	0.98	+ 0.012	41
					24.0	5.8	6.29	1.04	+ 0.012	42
					30.0	5.2	6.36	1.11	+ 0.010	46
					35.0	14.4	6.26	1.16	+ 0.006	41
					49.5			1.24		48
108	M	28	1.86	2960	— 14.0	5.4	5.69	1.11	— 0.006	74
					— 8.5	6.3	5.36	1.08	— 0.008	75
					— 2.0	18.4	5.51	1.03	+ 0.006	68
	50 gm glucose i. v. (0—14 min)			3067	16.0	5.4	5.86	1.14	± 0.000	63
					21.0	5.4	5.86	1.14	+ 0.028	57
					27.0	4.9	5.87	1.29	+ 0.008	62
					32.0	5.5	5.91	1.33	+ 0.016	59
					37.0	5.9	6.05	1.42	+ 0.010	58
					43.5	11.3	6.01	1.48	+ 0.006	54
					54.5					50
								1.55		
112	M	23	1.89	2967	— 10.5	5.3	5.81	1.11	+ 0.008	66
					— 5.5	4.9	5.75	1.15	+ 0.008	67
					— 0.5	10.5	5.55	1.19	— 0.012	65
	50 gm glucose i. v. (0—8.5 min)			3235	10.0	4.8	5.35	1.06	+ 0.010	58
					14.5	5.6	5.47	1.11	+ 0.013	59
					20.5	5.0	5.63	1.18	+ 0.014	57
					25.5	4.9	5.75	1.25	+ 0.012	54
					30.0	5.1	5.82	1.31	+ 0.016	54
					35.5	10.5	5.78	1.39	+ 0.008	53
					45.5			1.47		51

nic glucose data were conflicting. In five out of six experiments, glucose was released from the splanchnic area immediately after the injection. Thereafter, the results were inconsistent but a tendency towards a splanchnic glucose removal was seen during the later part of the experiments. The only subject with a splanchnic removal of glucose immediately after the glucose injection h a high basal splanchnic production of gluco It has been observed (Castenfors, Eliasch Hultman, to be published) that a liver wi high basal output of glucose is more "hu gry" for glucose and consequently will move substantial amounts of supplied gl cose.

rmal volunteers. Values are given for estimated splanchnic blood flow d splanchnic glucose production.

	A - Hv O_2 diff. ml/L	Splanchnic O_2 consumption ml/min	Hematocrit %	Hemoglobin extinction		Blood glucose mg %			Hv - A glucose diff. mg %	Glucose production mg/min
				Artery	Hep. vein	Artery	Hep. vein	Inferior caval vein		
10	—	—	40.5	.594	.599	81	88		7	
70	24.9	50.8	39.0	.585	.585	82	88		6	137.2
	—	—	38.0	.581	.586	81	87		6	118.2
10										271.4
10	27.3	53.5	35.5	.551	.553	387	408		21	
90	25.9	51.8	35.0	.557	.559	319	313		— 6	143.3
60	—	—	35.0	.563	.557	276	273		— 3	— 94.1
60	—	—	35.0	.562	.567	245	240		— 5	— 86.4
70	24.8	48.7	37.0	.558	.569	212	217		5	± 0.0
20	—	—	36.5	.560	.568	176	186		10	147.8
	—	—	37.0	.572	.570	153	146		— 7	24.7
00	—	—	41.0	.563	.558	87	104	81	17	
30	37.5	45.6	40.0	.561	.556	88	105	78	17	204.0
	—	—	40.0	.558	.563	91	105	79	14	190.7
50										281.3
20	38.1	50.9	40.0	.534	.544	361	392	332	31	
80	37.5	48.8	39.0	.525	.524	302	317	254	15	326.6
80	—	—	39.5	.533	.534	276	260	248	— 16	— 5.9
20	—	—	39.5	.535	.530	247	227	226	— 20	— 212.4
60	40.0	45.6	38.5	.538	.527	229	225	219	— 4	— 134.4
20	—	—	39.0	.537	.534	211	203	205	— 8	— 69.6
	—	—	39.0	.529	.528	170	172	179	2	— 36.6
90	—	—	42.0	.576	.559	84	96	82	12	
40	44.1	55.8	42.0	.580	.561	89	99	85	10	141.9
80	—	—	42.0	.567	.561	88	101	85	13	142.6
50	39.6	54.1	40.5	.537	.528	391	417	322	26	269.1
10	42.1	56.0	40.5	.539	.543	332	333	302	1	182.3
10	—	—	40.5	.535	.532	294	306	276	12	85.2
10	—	—	40.0	.536	.521	279	276	267	— 3	59.0
60	40.4	51.9	40.5	.542	.520	254	251	244	— 3	— 39.3
20	—	—	40.0	.541	.533	242	240	229	— 2	— 31.5
	—	—	40.0	.532	.545	219	219	210	± 0	— 12.5

The splanchnic glucose data after an intra-ous glucose injection reported earlier are o confusing:

. Soskin, Essex, Herrick & Mann (1938) nd in the dog a hepatic removal of glucose ing the first hour after an intravenous in-ion of a 30 per cent glucose solution (1.75 g glucose per kg body weight was given during 5 minutes).

2. Myers (1948) observed in man a marked increase in the splanchnic output of glucose immediately after a rapid intravenous injection (50 g glucose in a 10 per cent solution).

3. Bondy, James & Farrar (1949) found

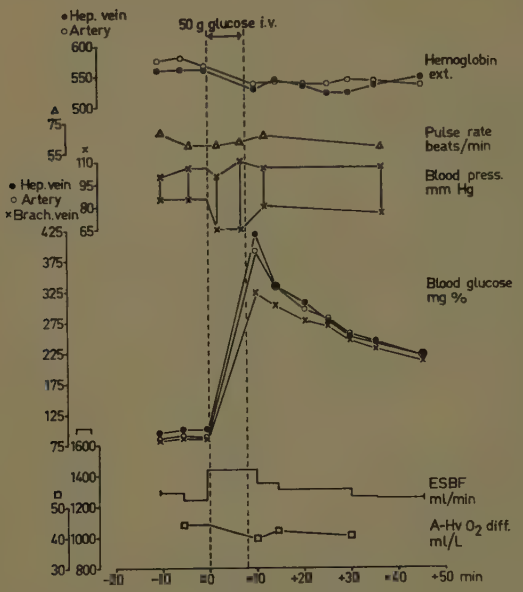


Fig. 1. A typical experiment with intravenous injection of 50 gm glucose on subject No. 112 (see Table I). The figure demonstrates the effect on ESBF, blood pressure and liver oxygen uptake.

in man an immediate retention of glucose by the liver after an intravenous injection of glucose (0.5 g glucose per kg body weight in 250 ml destilled water was given during 15 minutes).

4. Gordon, Craigie, Kerr, Cherrick & Sherlock (1960) observed in man on an average a negative glucose difference between 30 and 60 minutes from the time of injection (50 ml 50 per cent glucose was injected during 3 minutes). However, their results were not uniform and the Hv-A glucose differences were small.

It is difficult to explain the different results obtained by the above authors and in the present investigation. It is suggested that the variance is mainly due to the different rates of adminstration and the different concentrations of the injected glucose sulting in different rates of fall of the blo glucose concentration.

The mean value of the basal splanch glucose production was greater than th obtained by other investigators (Myers 19 Bondy *et al.* 1949, Bearn, Billing & Sherl 1951, and others). This was largely due the influence on the mean of the extrem high splanchnic glucose production in periment No. 82.

In two of the three experiments in wh the glucose concentration of the inferior ca vein was also measured, there were gr differences between the arterial and the ca blood indicating a peripheral removal of glucose given.

SUMMARY

The effect of a rapid intravenous inject of hypertonic glucose solutions on splanchnic blood flow and oxygen consu tion was studied in six healthy non-hospi ized volunteers.

The glucose injections resulted in a p nounced hyperglycemia and a moderate pansion of the plasma volume. The m blood glucose concentration decreased fr 370 mg glucose per 100 ml blood to 175 during the 45 minute period. No measura effect on either the splanchnic blood flow the splanchnic oxygen consumption found.

ACKNOWLEDGMENT

The authors wish to thank Miss Genév Leballeur, Miss Gill Robertson, Mrs Marga Söderling-Furu, Miss Inga Markgren and Ann-Marie Allgoth for technical assistance Mrs Ingrid Hallman for sercretary help.

REFERENCES

arn, A. G., Billing, B. H. & Sherlock, S.: Hepatic glucose output and hepatic insulin sensitivity in diabetes mellitus. Lancet *2*, 698, 1951.

ndy, P. S., James, D. F. & Farrar, B. W.: Studies of the role of the liver in human carbohydrate metabolism by the venous catheter technic. I. Normal subjects under fasting conditions and following the injection of glucose. J. Clin. Investigation *28*, 238, 1949.

adley, S. E., Ingelfinger, F. J., Bradley, G. P. & Curry, J. J.: Estimation of hepatic blood flow in man. J. Clin. Investigation *24*, 890, 1945.

andt, J. A., Castleman, L., Ruskin, H. D., Greenwald, J. & Kelly, J. J. Jr.: The effect of oral protein and glucose feeding on splanchnic blood flow and oxygen utilization in normal and cirrhotic subjects. J. Clin. Investigation *34*, 1017, 1955.

stenfors, H., Eliasch, H. & Hultman ,E.: The splanchnic blood flow and oxygen consumption estimated in man by the bromsulphalein method with special references to the influence of the peripheral dye level. Scandinav. J. Clin. & Lab. Investigation *12*, 158, 1960.

- Eliasch, H. & Hultman, E.: Dumpingsyndromets patofysiologi. Nord. med. *63*, 637, 1960.

Ek, J. & Hultman, E.: Determination of glucose and laevulose in body-fluids, Nature *181*, 780, 1958.

Elkinton, J. R. & Danowski, T. S.: The body fluids. p. 85. The Williams & Wilkins Company, Baltimore, 1956.

Gordon, E. E., Craigie, A., Kerr, D. N. S., Cherrick, G. R. & Sherlock, S.: Splanchnic uptake of glucose, inorganic phosphorus, and potassium after an intravenous glucose load in patients with cirrhosis and in subjects without liver disease. J. Lab. & Clin. Med. *55*, 829, 1960.

Hultman, E.: Rapid specific method for determination of aldosaccharides in body fluids. Nature *183*, 108, 1959.

Myers, J. D.: The effect of the intravenous administration of glucose and amino acids on the hepatic blood flow and splanchnic oxygen consumption of man. Federation Proc. *7*, 83, 1948.

— Net splanchnic glucose production in normal man and in various disease states. J. Clin. Investigation *29*, 1421, 1950.

Soskin, S., Essex, H. E., Herrick, J. F. & Mann, F. C.: The mechanism of regulation of the blood sugar by the liver. Am. J. Physiol. *124*, 558, 1938.

Scandinav. J. Clin. & Lab. Investigation 512—524, 13, 1961.

EFFECTS OF INGESTION OF HYPEROSMOTIC GLUCOSE SOLUTION ON THE SPLANCHNIC CIRCULATION IN NORMA SUBJECTS AND IN PARTIALLY GASTRECTOMIZED PATIENT REACTING WITH CIRCULATORY COLLAPSE[1]

By H. CASTENFORS, H. ELIASCH and E. HULTMAN

From the Central Laboratory, the 2nd Medical Service and the Roentgen Department, S:t Erik Hospital and the Department of Medicine, the Serafimer Hospital, Stockholm, Sweden

(Received for publication March 1, 1961)

A considerable drawback in the surgical therapy of peptic ulcer is serious postgastrectomy syndrome (dumping) appearing in 5 to 10 per cent of patients submitted to partial gastrectomy. It is well known that during a dumping attack, signs of vasomotor disturbance occur. Many investigators consider this circulatory unbalance to be a consequence of a decrease in the plasma volume (Roberts, Randall, Farr, Kidwell, McNeer & Pack 1954, Amdrup & Jørgensen 1957, Wallensten 1960, among others). However, others have not been able to correlate the degree of decrease in the plasma volume with the severity of symptoms (Hinshaw, Joergensen, Davis & Stafford 1957, Duthie, Irvine & Kerr 1959 and Borgström 1960, among others). This lack of correlation has been thought to be due to a varying individual sensitivity to loss of plasma wa (Le Quesne, Hobsley & Hand 1960) or a concomitant increase of the periphe blood flow (Hinshaw *et al.* 1957). On other hand it has been suggested that dumping reaction is caused by a rele adrenaline (Smith 1951 and Duthie *et* 1959, among others) and also that there an accumulation of blood in the veins of splanchnic area (Hoffmann 1939 and Sch de & Heinecker 1955).

In the face of these diverging concept was thought timely to study splanch hemodynamics during the dumping epis in partially gastrectomized patients. dumping attack was provoked by giv a hyperosmotic glucose solution orally. to the type of technique used, most pati went into a circulatory collapse after a sh while. In this paper results will be prese from seven collapse studies. Control stu on healthy human subjects will also be sented. When the same technique was ployed these healthy subjects did not

[1] The work described in this paper was aided by grants from the Swedish National Association Against Tuberculosis and from the Swedish Institute for Investigations on Malt Beverages.

ience any discomfort. It was found that marked decrease in splanchnic vascular istance preceeded the circulatory collapse the gastrectomized patients, while in the rmal subjects no corresponding change s observed.

MATERIAL

Seven non-hospitalized healthy volunteers with- history of significant gastric complaints and en partially gastrectomized individuals were lied. Physical data are given in the tables. One ent, No. 106, had no history of dumping reac- and had full working capacity. The other six e hospitalized for dumping symptoms and had ced working capacity. All had been partially trectomized with gastro-jejunostomy according he Polya method several years before the pre- investigation; one patient, No. 91, had also submitted to a vagal resection.

METHODS

The studies were performed in the morning. The ents and the volunteers had fasted since the going evening. 0.1 gm of sodium amytal was en orally 30 minutes before the start of the y. With the individuals in the supine position left femoral vein was punctured and, under roscopic control, a polythene catheter intro- ed percutaneously and passed *via* the inferior al vein into the right hepatic vein (Castenfors, sch & Hultman 1960 A). A small polythene eter was then placed in a cubital vein and ther in one of the brachial arteries. After this cedure the subjects were carried from the X-ray e to a comfortable chair in which they sat ughout the study. The splanchnic blood flow estimated by use of the BSP (bromsulphalein) hod of Bradley, Ingelfinger, Bradley & Curry 45).

A priming dose of 100 mg of BSP was first cted. Thereafter the BSP infusion was given constant rate with a motor-driven syringe (Ek 5). The flask containing the BSP solution was ed on an automatic balance allowing continuous registration of the quantities infused. Approximately 3 mg BSP per square meter body surface area was infused per minute. After 30 minutes of infusion (equilibration time) sampling was started. Three sets of simultaneous blood samples, 5 to 10 minutes apart, were drawn from the hepatic vein and the brachial artery for the control data. When these samples were obtained the subjects drank 400—500 ml of a 20 per cent glucose solution in 1 to 5 minutes. The solution had a temperature of 37° C. After this administration, simultaneous samples from the hepatic vein and the brachial artery were drawn at varying intervals during 20 to 50 minutes. The blood pressure (cuff) and the pulse rate (palpatory) were continuously measured throughout the experiment. The patients were tilted to a horizontal position when signs of circulatory collaps appeared or were manifest. In four studies sampling was continued in this position.

The analytical methods have been previously described for BSP, hemoglobin and oxygen (Castenfors *et al.* 1960 A) and sugar (Hultman & Castenfors 1961). The hematocrit values were determined with a Wifug centrifuge.

CALCULATIONS

$$\text{Splanchnic removal} = I \pm \frac{\Delta P}{100} \times \text{Extrasplanchnic BSP space}$$

$$\text{Estimated splanchnic blood flow (ESBF)} = \frac{\text{Splanchnic removal}}{(p—h) \times \frac{100—Hct}{100}}$$

The extrasplanchnic BSP space was assumed to be 0.8 times the total BSP space (Bradley, Marks, Reynell & Meltzer 1953). Changes in plasma volume (BSP space) and in blood volume were estimated by employing the following formulae:

$$B_1 \times hb_1 = B_2 \times hb_2; \; B_2 = B_1 \times \frac{hb_1}{hb_2}$$

$$\text{BSP space}_2 = \text{BSP space}_1 \times \frac{hb_1\,(100—Hct_2)}{hb_2\,(100—Hct_1)}$$

The plasma volume (BSP space) was computed

from the bodyweight according to the formula of Sjöstrand (1949). When the BSP space changed during the clearance period the removal was calculated from: Splanchnic removal =

$$= I \pm \frac{\text{Extrasplanchnic BSP space}_1 \times p_1 - \text{Extrasplanchnic BSP space}_2 \times p_2}{t}$$

This formula is valid only if changes in the BSP space are parallel inside and outside the splanchnic area.

Estimated splanchnic oxygen consumption (ESC ml/min = ESBF, L/min × Splanchnic oxy difference, ml/L

I	= infusion rate, mg/min
p	= arterial plasma concentration of BSP, mg/100 ml plasma
h	= hepatic vein plasma concentration of BSP mg/100 ml plasma
Δp	= change in p in mg/100 ml plasma per min
Hct	= hematocrit, per cent
t	= period time, minutes (t_2—t_1)
$BSP\ space_1$, $BSP\ space_2$	= BSP space at t_1, t_2, ml
p_1, p_2	= BSP arterial plasma concentration at t_1, t_2, mg/100 ml plasma
B_1, B_2	= blood volume at t_1, t_2
hb_1, hb_2	= hemoglobin concentration at t_1, t_2, extinction
Hct_1, Hct_2	= hematocrit value at t_1, t_2, per cent.

RESULTS

I. *Control values in the sitting position.*

Pertinent data are listed in Table I. Seven healthy men, three gastrectomized women and four gastrectomized men were studied. The normal men had on an average a lower splanchnic oxygen difference than the male patients but the mean ESBF was almost the same in both groups. The gastrectomized women had a lower mean ESBF and a greater splanchnic oxygen difference than the male patients as well as the normal subjects; the difference was not significant.

II. *The effect of the glucose test meal.*

A. *Normal subjects.* Table II, Fig. 1.

The seven normal subjects showed no sign discomfort after the test meal; they sat comfort in the chair throughout the experiment. The ob vation period ranged between 25 and 46 min The results of the circulatory response to the meal are reported in the table as the means of maximal change in pulse rate, blood volume, ES and A-HvO_2 difference, respectively.

a. *Pulse rate, blood volume, arterial blood pressure.*

In the normal subjects the glucose meal followed by a rise in pulse rate, the blood pres

Table I. *Control values in the sitting position.*

	ESBF ml/min	A-HvO_2 diff. ml/L
Seven normal males	1510 (1100—1990)	41.1 (26.5—60.8)
Four partially gastrectomized males	1410 (1085—2010)	46.4 (38.9—61.1)
Three » » females	1010 (655—1485)	60.7 (52.7—69.7)

ıg essentially constant. The maximal increase in se rate averaged 8 beats per minute. The blood ume tended to decrease slightly (0.7—5.9 per t) during the first 30 minutes after the meal. In subjects this decrease was followed by an in- ase of the blood volume towards the end of the dy (Nos. 23, 24, 25, 27, and 31). The appearance e for peak values in blood volume and pulse e varied widely during the period of observation.

Estimated splanchnic blood flow (ESBF).

The ESBF changed slightly in four studies and reased markedly in three. The two types of re- ion are illustrated in Fig. 1. The maximal in- ase in the ESBF averaged 380 ml per minute or per cent, appearing 19 minutes after the meal.

Arterio-hepatic venous oxygen difference ($A\text{-}HvO_2$), and estimated splanchnic oxygen consumption (ESOC).

The $A\text{-}HvO_2$ difference did not change signi- antly in five out of seven studies. In the two remaining studies there was a successive decrease; in these the highest resting $A\text{-}HvO_2$ difference values were encountered.

Due to the variations in the $A\text{-}HvO_2$ difference, the estimated splanchnic oxygen consumption fluctuated markedly after the meal and decreases as well as increases were observed.

d. *Glucose resorption.*

After the meal the blood glucose concentration rose steadily throughout the experiment.

In all subjects the glucose concentration was higher in the hepatic vein than in the artery but this difference tended to decrease towards the end of the study. The highest values in arterial and hepatic venous glucose concentration averaged 200 and 219 mg glucose per 100 ml blood, respectiviely. However, the real peak values were never observed since the blood glucose concentration was still rising when the studies were discontinued. In order to estimate the speed of resorption, the control arterial concentration was subtracted from the arterial concentration at 10 and 25 minutes after the glucose meal, respectively. These values are given in Table III.

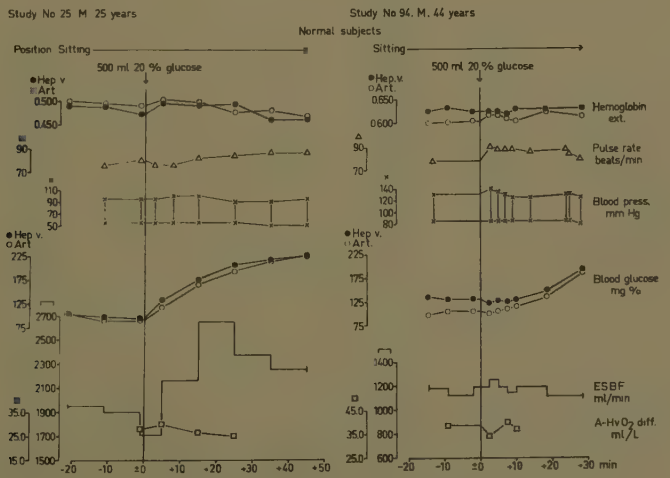


Fig. 1. The effect of oral glucose ingestion on different parameters in two healthy volunteers.

Table II. *The effect of the glucose meal in seven healthy volunteers. Figures of maximal deviation in the different parameters.*

Study No.	Sex Age BSA M^2		Time min	E S B F ml/min	Change ml/min	Change %	A-HvO^2 diff. Time min	ml/l	Change ml/l	Ch
23	M	Before glucose		1925				36.1		
	35	After glucose								
	2.00	max.	16.0–26.0	2860	+ 935	+ 48.6	16.0	36.4	+ 0.3	+
		min.					6.0	40.7		
		0′–16′max.	6.0–16.0	2100	+ 175	+ 9.1	16.0	36.4	+ 0.3	+
								43.7		
24	M	Before glucose		1245						
	38	After glucose								
	1.65	max.	26.0–36.0	1340	+ 95	+ 7.6	16.0	47.1	+ 3.4	+
		min.					6.0	56.1		
		0′–16′max.	6.0–16.0	1170	— 75	— 6.0	16.0	47.1	+ 3.4	+
				1925				28.0		
25	M	Before glucose								
	25	After glucose								
	1.83	max.	15.0–25.0	2650	+ 725	+ 37.7	25.0	24.9	— 3.1	—
		min.					5.0	29.8		
		0′–16′max.	5.0–15.0	2160	+ 235	+ 12.2	15.0	26.3	— 1.7	—
				1100				60.8		
27	M	Before glucose								
	41	After glucose								
	2.17	max.	6.0–16.0	1580	+ 480	+ 43.6	26.0	41.8	— 19.0	—
		min.					6.0			
		0′–16′max.	6.0–16.0	1580	+ 480	+ 43.6	16.0	46.0	— 14.8	—
				1255				54.2		
31	M	Before glucose								
	29	After glucose								
	2.08	max.	20.0–25.0	1620	+ 365	+ 29.1	6.0	44.6	— 9.6	—
		min.					15.0			
		0′–16′max.	10.0–15.0	1510	+ 255	+ 20.3	6.0	44.6	— 9.6	—
				1150				38.5		
94	M	Before glucose								
	44	After glucose								
	2.03	max.	2.5–5.0	1250	+ 100	+ 8.7	2.5	33.9	— 4.6	—
		min.					7.5	39.8		
		0′–16′max.	2.5–5.0	1250	+ 100	+ 8.7	2.5	33.9	— 4.6	—
				1990				26.5		
98	M	Before glucose								
	36	After glucose								
	2.14	max.	20.0–30.0	1950	— 40	— 2.1	6.0	25.0	— 1.5	—
		min.					13.5	32.1		
		0′–16′max.	3.0–6.0	1820	— 170	— 8.5	6.0	25.0	— 1.5	—
	Before glucose:									
		n		7				7		
		mean		1513				41.1		
	After glucose	max.								
		n	7	7	7	7	7	7	7	
		mean	19	1893	+ 380	+ 24.7	14	36.2	— 4.9	—
	0′–16′ max:									
		n	7	7	7	7	7	7	7	
		mean	9	1656	+ 143	+ 11.3	11	37.0	— 4.1	—
		S. E.			± 82				± 2.4	

Table II. *(forts.)*

E S O C			Blood volume			Pulse rate				Amount 20 % glucose given orally
ml/min	Change ml/min	Change %	Time min	Art. Hb ext.	Change %	Time min	Beats /min	Change beats/min	Change %	
69.7				0.609			66			
90.3	+ 20.6	+ 29.6	6.0	0.617	— 1.3	6.0	68	+ 2	+ 3.0	500
79.6			36.0	0.599	+ 1.7					
90.3	+ 20.6	+ 29.6	6.0	0.617	— 1.3	6.0	68	+ 2	+ 3.0	
53.8				0.511			97			
57.0	+ 3.2	+ 5.9	26.0	0.523	— 2.3	36.0	114	+ 17	+ 17.5	460
67.3			46.0	0.509	+ 0.4					
57.0	+ 3.2	+ 5.9	16.0	0.513	— 0.4	16.0	110	+ 13	+ 13.4	
53.2				0.495			77			
62.5	+ 9.3	+ 17.5	5.0	0.502	— 1.4	35.0	86	+ 9	+ 11.7	500
57.7			45.0	0.467	+ 6.0					
63.3	+ 10.1	+ 19.0	5.0	0.502	— 1.4	15.0	82	+ 5	+ 6.5	
71.1				0.523			75			
50.6	— 20.5	— 28.8	6.0	0.536	— 2.4	16.0	76	+ 1	+ 1.3	500
72.9			46.0	0.512	+ 2.1					
61.9	— 9.2	— 12.9	6.0	0.536	— 2.4	16.0	76	+ 1	+ 1.3	
67.2				0.611			88			
62.7	— 4.5	— 7.2	6.0	0.615	— 0.7	15.0	92	+ 4	+ 4.5	500
77.6			25.0	0.585	+ 4.4					
62.7	— 4.5	— 7.2	6.0	0.615	— 0.7	15.0	92	+ 4	+ 4.5	
44.3				0.602			78			
41.4	— 2.9	— 6.5	18.5	0.626	— 3.8	2.5	90	+ 12	+ 15.4	500
46.4										
41.4	— 2.9	— 6.5	2.5	0.618	— 2.6	2.5	90	+ 12	+ 15.4	
52.7				0.547			64			
41.9	— 10.8	— 20.5	13.5	0.581	— 5.9	20.0	72	+ 8	+ 12.5	500
50.9										
41.9	— 10.8	— 20.5	13.5	0.581	— 5.9	6.0	68	+ 4	+ 6.3	
7							7			
58.9							78			
7	7	7	7		7	7	7	7	7	
58.1	— 0.8	— 1.4	12		— 2.5	19	85	+ 7.6	+ 9.4	
7	7	7	7		7	7	7	7	7	
59.8	+ 0.9	+ 1.1	8		— 2.1 ± 0.7	11	84	+ 6 ± 2	+ 7.2	

Table III. *The increase in arterial blood glucose concentration in normals and patient*

	10 min increase in art. blood glucose conc. mg %	25 min increase in art. blood glucose conc. mg %
Normals:		
n	7	7
mean	+ 24	+ 81
S. E.	± 6.9	± 10.1
Patients:		
n	7	4
mean	+ 54	+ 104
S. E.	± 9.1	± 18.0

B. *Partially gastrectomized patients.* Table IV, Fig. 2.

All these patients reacted with a fall in arterial blood pressure resulting in a circulatory collapse.

Immediately after they had drunk the glucose they started to complain of epigastric pain, dizziness and fatigue. Varying between 11 and 17 minutes (mean 13) from the onset of the glucose ingestion the heart rate decreased and the blood pressure fell; the patients went into a semiconscious state. They were immediately tilted to horizontal position where the blood pressure was normalized and the symptoms diminished.

All the circulatory parameters recorded during the first minutes after the meal followed a typical course which was interrupted by the pressure fall. In order to obtain representative values during the "precollapse" time, the means of the maximal deviation in all variables prior to the blood pressure fall were computed. These means were compared to the corresponding means obtained in the healthy subjects, *i. e.* the maximal changes during the first 16 minutes after the test meal in the latter studies (Table I).

a. *Pulse rate, blood volume, arterial blood pressure.*

Immediately after the meal the pulse rate increased, reaching a maximal value before the collapse, then, suddenly dropped and increased again after the tilting when the blood pressure rose normal (Figs. 3 and 4). The maximal incre averaged 21 beats per minute (S.E. 3.5) and significantly greater than the corresponding m value of the normal subjects ($0.01 > p > 0.001$).

The blood volume decreased rapidly after meal, reaching its lowest value immediately bef

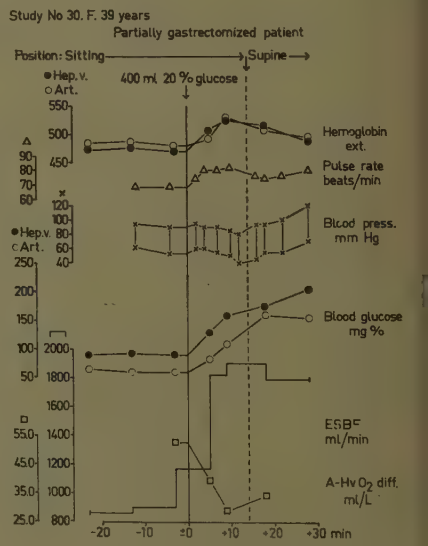
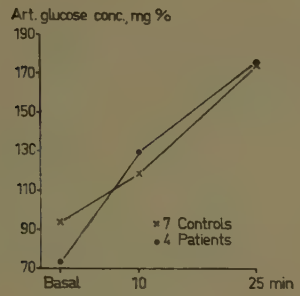


Fig. 2. The effect of oral glucose ingestion different parameters in a partially gastrectom patient reacting with a marked fall in blood pr ure and subsequent syncope.



g. 3. The arterial blood glucose concentration in ven healthy volunteers and in four partially gast-ctomized patients. Mean values before and after the glucose meal.

during the collapse. In three out of four studies here blood was sampled after the collapse, the ood volume rose successively after the patients d been tilted to the horizontal position. The aximal decrease during the precollapse time aver-ged 6.6 per cent (S.E. 1.2); this decrease was gnificantly greater than the corresponding mean r the normal individuals ($0.01 > p > 0.001$). The emoglobin concentration of the hepatic venous ood was not significantly higher than the hemo-obin concentration of the arterial blood after the ucose meal.

The estimated splanchnic blood flow (ESBF).

After the glucose meal the ESBF increased pidly in all seven patients. The BSP data showed greater removal and a lower extraction of BSP. he mean of the maximal ESBF value prior to the ood pressure fall was calculated. Two studies Nos. 26 and 28) were excluded because in these xperiments the last sample of the clearance was awn after the onset of the collapse and, hence, e maximal ESBF value in these two patients was obably not representative of the precollapse phase. the other five patients, sampling intervals were orter and the samples were obtained before the llapse. The average maximal increase in ESBF these five patients was 722 ml per minute (70 per nt) (S.E. 152). This increase was significantly gher than the corresponding increase in the normal subjects (0—16 minutes) ($0.01 > p > 0.001$). In four patients (Nos. 26, 28, 30, and 41) the ESBF was determined after tilting to the horizontal position in which the blood pressure had returned to normal values. The ESBF was moderately lower in this position as compared to the corresponding maximum before the fall in blood pressure (Table IV).

c. *Arterio-hepatic venous oxygen difference ($A\text{-}HvO_2$) and the estimated splanchnic oxygen consumption (ESOC).*

After the glucose administration, the $A\text{-}HvO_2$ difference decreased in all studies and in six the negative peak was observed before the drop in blood pressure. In the seventh study (No. 28) the $A\text{-}HvO_2$ difference fell markedly (69.7 to 27.8 ml/L) during the precollapse time but a further decrease to 24.5 ml/L was observed after tilting to the horizontal position. Due to the long sampling intervals the minimum value was probably not ob-

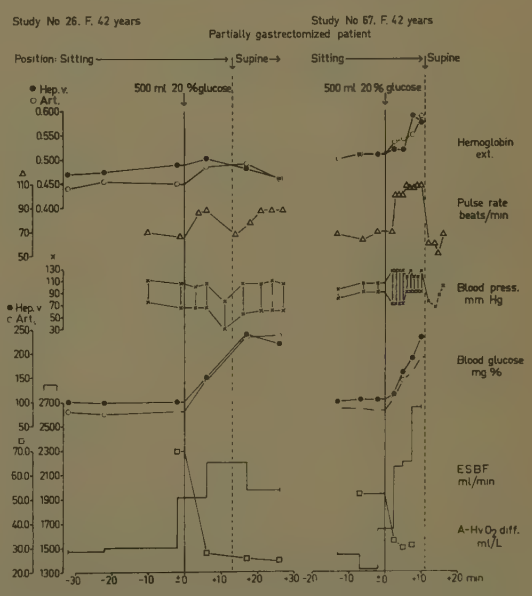


Fig. 4. Two experiments on a partially gastrectomized patient. The control study with more frequent blood sampling shows better correlation between the ESBF and the $A\text{-}HvO_2$ difference.

Table IV. *The effect of the glucose meal in seven partially gastrectomized patients. Figures of maximal deviation in the different parameters. +) The clearance period includes part of the collapse phase.*

Study No.	Sex Age BSA M^2		ESBF Time min	ESBF ml/min	ESBF Change ml/min	ESBF Change %	A-HvO^2 diff. Time min	A-HvO^2 diff. ml/l	A-HvO^2 diff. Change ml/l	A-HvO^2 diff. Cha %
26	F	Before glucose		1485				69.7		
	41	After glucose								
	1.62	max.	6.0–17.0	2200+)			6.0	27.8	— 41.9	—
		min.	17.0–26.0	1970	+ 485	+ 32.7	26.0	24.5	— 45.2	—
28	M	Before glucose		2010				42.7		
	29	After glucose								
	1.86	max.	7.0–17.0	2250+)			7.0	27.6	— 15.1	—
		min.	27.0–37.0	1960	— 50	— 2.5	27.0	47.3	+ 4.6	+
30	F	Before glucose		880				52.7		
	39	After glucose								
	1.69	max.	5.0–9.0	1820	+ 940	+ 106.8	9.0	29.1	— 23.6	—
		min.	18.0–28.0	1790			18.0	34.6	— 18.1	—
41	M	Before glucose		1085				43.0		
	32	After glucose								
	1.74	max.	5.0–8.0	1570	+ 485	+ 44.7	12.0	28.7	— 14.3	—
		min.	22.0–31.0	1480				–		
91	M	Before glucose		1125				38.9		
	36	After glucose								
	1.89	max.	6.0–8.0	1590	+ 465	+ 41.3	4.5	29.3	— 9.6	—
93	F	Before glucose		655				59.8		
	53	After glucose								
	1.78	max.	6.0–8.5	1160	+ 505	+ 77.1	6.0	47.8	— 12.0	—
106	M	Before glucose		1425				61.1		
	40	After glucose								
	1.99	max.	6.0–9.0	2640	+ 1215	+ 85.3	6.0	49.6	— 11.5	—
	Before glucose:									
		n		5				7		
		mean		1034				52.6		
	After glucose max:									
		n		5	5	5	7	7	7	
		mean	7	1756	+ 722	+ 71.0	7	34.3	— 18.3	—
		S.E.			— 152				+ 4.3	

tained in some studies. At, or immediately before the collapse, the A-HvO_2 difference returned to the resting values (Fig. 2). The maximal decrease in the seven studies during the precollapse phase averaged 18.3 ml/L (35 per cent) (S.E. 4.3) and was significantly higher than the corresponding mean of the decrease obtained in the normal subjects ($0.02 > p > 0.01$). The magnitude of the decrease in the A-HvO_2 difference was not correlated to the increase in the ESBF; however, peak values in both variables appeared at approximately the same time and subsequent changes were parallel

Table IV. *(forts.)*

ESOC			Blood volume			Pulse rate				Amount 20 % glucose given orally
ml/min	Change ml/min	Change %	Time min	Art. Hb ext.	Change %	Time min	Beats /min	Change beats/min	Change %	
104.6				0.448			68			
57.1*)			6.0	0.484	— 7.4	6.0	88	+ 20	+ 29.4	500
48.3	— 56.3	— 53.8	26.0	0.462	— 3.0	17.0	78	+ 10	+ 14.7	
86.3				0.589			67			
60.7*)			7.0	0.608	— 3.1	7.0	92	+ 25	+ 37.3	500
88.0	+ 1.7	+ 2.0	37.0	0.586	+ 0.5	27.0	80	+ 13	+ 19.4	
47.4				0.484			68			
54.1	+ 6.7	+ 14.1	9.0	0.529	— 8.5	9.0	82	+ 14	+ 20.6	400
63.8	+ 16.4	+ 34.6	28.0	0.495	— 2.2	18.0	74	+ 6	+ 8.8	
44.7				0.561			77			
40.9	— 3.8	— 8.5	12.0	0.591	— 5.1	12.0	105	+ 28	+ 36.4	500
–			35.0	0.589	— 4.8	22.0	88	+ 11	+ 14.3	
43.6				0.642			61			
41.0	— 2.6	— 6.0	15.0	0.732	— 12.3	6.0	96	+ 35	+ 57.4	500
38.9				0.505			100			
50.9	+ 12.0	+ 30.8	11.0	0.539	— 6.3	11.0	120	+ 20	+ 20.0	450
87.1				0.600			65			
123.0	+ 35.9	+ 41.2	14.5	0.623	— 3.7	6.0	72	+ 7	+ 10.8	396
5							7			
52.3							72			
5	5	5	7		7	7	7	7	7	
62.0	+ 9.6	+ 14.3	11		— 6.6 ± 1.2	8	94	+ 21 ± 3	+ 30.3	

To get a rough estimate of the splanchnic oxy- consumption (ESOC) during the precollapse se, the minimal A-HvO_2 difference was multip- by its corresponding ESBF value. The mean hese products in five patients (Nos. 26 and 28 luded) was 62.0 ml per minute, and the corres- ding control mean was 52.3 ml per minute erage increase: 14 per cent).

d. *Glucose resorption.*

The glucose concentration of the arterial and hepatic vein blood rose rapidly after the glucose administration during the precollapse phase. The ten minutes' increase in arterial blood glucose concentration was significantly higher than the corresponding increase in the normal subjects ($p \approx 0.02$) (Table III). In Fig. 3 the mean curve of the

normalsubjects is given together with the mean curve of four patients who were also studied after the pressure fall. It is seen that the increase was more rapid in the patients during the first 10 minutes. Thereafter, the reverse relationship was present the 25-minute values being almost equal in both groups.

DISCUSSION

It has long been known that the dumping episode in the partially gastrectomized patient is associated with vascomotor reactions of varying degrees. While considerable interest has been directed towards the observed changes in the total blood volume, no studies concerning splanchnic hemodynamics seem to have been reported. Since the vasomotor reactions in the dumping episode occur in close connection to the ingestion of a meal, it seemed conceivable that one of the primary reactions would be a change in the blood flow of the splanchnic area.

In seven out of nine gastrectomized patients ingestion of 500 ml 20 per cent glucose solution resulted in a circulatory collapse. This high incidence of circulatory collapse is a finding in contrast to the experience of other workers generally using a solution of 100—150 ml of 50 per cent glucose. The reason for the different reaction can be that a larger volume of fluid and greater amount of glucose was given in these series and also that the present studies include the procedure of hepatic venous catheterization.

A preliminary report on a part of the present results has been published earlier (Castenfors, Eliasch & Hultman 1960 B). In the present paper only patients reacting with circulatory collapse are presented. The remaining two patients, showing essentially the same splanchnic circulatory response, will b included in a subsequent paper (Castenfors)

During the first 11—17 minutes after th glucose ingestion, the patients responde with a marked increase in the splanchni blood flow. The validity of the observed in crease in the splanchnic blood flow may however, be questioned. The flow was est mated by the BSP method, which is a clea ance procedure that renders mean values fo the period of determination. This means tha at rapid changes in blood flow, erroneou values may be encountered. This is show in Fig. 4, where the period of determinatio included a part of the collapse state, leadin to a falsely low value. However, in most o the present studies this difficulty was dimin shed by using short clearance periods.

The measurement of the splanchnic bloo flow necessitates determination of the arteri hepatic venous BSP difference and calcul tion of the splanchnic removal of the dy The arterio-hepatic venous difference measured directly by catheterization of th hepatic vein. The splanchnic removal BSP is estimated indirectly; at a consta arterial dye concentration this removal assumed to be equal to the infused amou of dye. When the splanchnic blood flow subject to alteration, as in the present stud a change in the arterial concentration BSP can appear. In this circumstance, t splanchnic removal is not equal to the i fused amount and must consequently be co rected for the change in the amount of d contained in the extrasplanchnic vascul space. The validity of such corrections w studied in an earlier investigation (Caste fors, Eliasch & Hultman 1961) and it w found that only small errors in ESBF we

ıcountered, even in the face of large varia-
ons in the peripheral concentration of dye.
hese studies were made on resting subjects
ıd it was thus assumed that the splanchnic
ood flow as well as the extrasplanchnic
ood volume were essentially unchanged.
the extrasplanchnic plasma volume is
ıanging this must be corrected for in the
ılculation of the splanchnic removal of dye.
ı the present study the changes in the ex-
asplanchnic space were calculated from
oserved changes in the hemoglobin conc.
ıd hematocrit value. A possible redistribu-
on of the plasma volume ("splanchnic pool-
ıg") could not be measured and, thus, not
ken into account. In all patients the oral
lucose ingestion resulted in an increased
SP clearance together with a decreased
olanchnic extraction ratio of dye. This can
ardly be explained by anything but an in-
reased splanchnic blood flow. Consequently
ıere can scarcely be any doubt that the
olanchnic blood flow did increase in the
xperiments reported here. This concept is
so supported by the finding of a concomi-
ınt decrease in the arterio-venous oxygen
ifference over the splanchnic area.

The ingestion of glucose resulted in an in-
rease of the blood glucose concentration.
he hyperglycemia *per se* could not be the
ıuse of the increase in blood flow as it has
ecently been demonstrated (Hultman *et al.*
961) that a high blood glucose concentra-
on, induced by intravenous injections of
ypertonic glucose solutions, does not give
ıy significant change in either the splanch-
ic blood flow or the splanchnic oxygen
onsumption. This is also supported by the
ct that in the control studies on normal
ıbjects in the present investigations high blood glucose levels could be present without significant change in the splanchnic blood flow.

In the gastrectomized patients the marked increase in the splanchnic blood flow appeared 11—17 minutes after the glucose ingestion (precollapse time). This increase in blood flow was significantly higher than that observed in the normal subjects during the same period of time. The rise in flow, associated with a decrease in the blood volume, was parallelled by an immediate and sustained increase in the pulse rate, until circulatory collapse ensued. Available data do not permit any conclusions as to the cause of this marked circulatory reaction. However, upright posture, decrease in vascular resistance, and reduction in blood volume must be factors of importance.

SUMMARY

1. The splanchnic blood flow, the splanchnic arterio-venous oxygen and glucose difference, as well as changes in the total blood volume were repeatedly measured in seven normal subjects and seven partially gastrectomized patients.

2. The subjects and the patients were studied in the sitting position. Values were obtained at rest and following the oral ingestion of 500 ml of a 20 per cent glucose solution.

3. The glucose administration caused no untoward effects in the normal subjects. All patients reacted with a fall in blood pressure and subsequent syncope.

4. A marked increase in the splanchnic blood flow and a moderate decrease in the total blood volume was observed in the pa-

tients, while the corresponding changes in the normals were small and inconsistent.

5. The validity of the observed increase in the splanchnic blood flow is discussed.

ACKNOWLEDGEMENT

The authors wish to thank Mrs. Margareta Söderling-Furu, Miss Geneviève Leballeur, Miss Gill Robertson, Miss Inga Markgren, Mrs. Ann-Marie Allgoth and Miss Inga Landbrink for technical assistance and Mrs. Ingrid Hallman for secretarial help.

REFERENCES

Amdrup, E. & Jørgensen, J. B.: Variations in the plasma volume occuring during "dumping" attacks. Acta chir. Scandinav. *112,* 294, 1957.

Borgström, S. G.: The efferent loop dumping syndrome and its relation to intestinal absorption as studied by an intubation technique. Acta chir. Scandinav. Suppl. 265, pp. 104, 1960.

Bradley, S. E., Ingelfinger, F. J., Bradley, G. P. & Currey, J. J.: Estimation of hepatic blood flow in man. J. Clin. Investigation *24,* 890, 1945.

— Marks, P. A., Reynell, P. C. & Meltzer, J.: The circulating splanchnic blood volume in dog and man. Ass. of Amer. physicians, Transactians *66,* 294, 1953.

Castenfors, H.: To be published.

— Eliasch, H., Hultman, E.: The splanchnic blood flow and oxygen sumption estimated in man by the bromsulphalein method with special reference to the influence of the peripheral dye level. Scandinav. J. Clin. & Lab. Investigation *12,* 158, 1960 A.

— — — Dumpingsyndromets klinik, patofysiologi och behandling. Nord. med. *63,* 637, 1960 B.

— — — Estimation of the splanchnic blood flow in man by the bromsulphalein (BSP) method; the effect of continuous variation of the peripheral plasma concentration of BSP. Scandinav. J. Clin. & Lab. Investigation *13,* 489, 1961.

Duthie, H. L., Irvine, W. T. & Kerr, J. W.: Cardiovascular changes in the postgastrectomy syndrome. Brit. J. Surg. *46,* 350, 1959.

Ek, J.: The influence of heavy hydration on the renal function in normal and hypertensive man. Scandinav. J. Clin. & Lab. Investigation Suppl. 19, 1955.

Hinshaw, D. B., Joergensen, E. J., Davis, H. A. Stafford, C. E.: Peripheral blood flow and blood volume studies in dumping syndrome. A.M.A. arch. Surg. *74,* 686, 1957.

Hoffmann, V.: Klinische Krankheitsbilder nach Magenoperationen. München. med. Wchnsch. *86,* 332, 1939.

Hultman, E. & Castenfors, H.: Effect of injection of hypertonic glucose on splanchnic blood flow and oxygen consumption. Scandinav. J. Clin. & Lab. Investigation *13,* 1961.

Le Quesne, L. P., Hobsley, M. & Hand, B. H. The dumping syndrome. I. Factors responsible for the symptome. Brit. M. J. *1,* 141, 1960.

Roberts, K. E., Randall, H. T., Farr, H. W., Kickwell, A. P., McNeer, G. P. & Pack, G. T. Cardiovascular and blood volume alteration resulting from intrajejunal administration of hypertonic solutions to gastrectomized patients: the relationship of these changes to the dumping syndroma. Annals of Surg. *140,* 631, 195

Schrade, W. & Heinecker, R.: Alimentäre Kreislaufstörungen als Ursache des sogenannten Dumping-Syndroms. Schweiz. med. Wchnsch. *85,* 481, 1955.

Sjöstrand, T.: The total quantity of hemoglobin in man and its relation to age, sex, bodyweight and height. Acta physiol. Scandinav. *18,* 32 1949.

Smith, W. H.: Potassium lack in the post-gastrectomy dumping syndrome. Lancet *261,* 745, 195

Wallensten, S.: The dumping syndrome. III. Conversion of Billroth II to Billroth I gastrectomy for severe dumping syndrome. Acta chir. Scandinav. *118,* 278, 1960.

Technique and Practical Problems

candinav. J. Clin. & Lab. Investigation 525—528, 13, 1961.

A SIMPLE TECHNIQUE FOR CONCENTRATING GRANULOCYTES FOR THE DETERMINATION OF CHROMATIN SEX

By A. DE LA CHAPELLE

From the Minerva Institute for Medical Research, Helsingfors, Finland.

(Received for publication December 23, 1960)

Barr & Bertram (1949) made the sensa-onal discovery that female intermitotic cells ntained sex chromatin bodies. Simple and liable methods for determining the genetic x from the chromatin bodies have been veloped (Moore & Barr 1955, Klinger & idwig 1957). The determination of the sex romatin from oral mucosa cells is a wide-read and reliable method.

The discovery of Davidson & Smith 954) that granulocytes from peripheral ood can be used to determine the sex romatin provided a simple way of checking e result obtained with oral mucosa cells. ne leukocyte technique, which has been stematically worked out by Kosenow & upin (1956), seems to be considered the ethod of choice by some workers, *e. g.* iedemann (1959). Among workers dealing th human chromosomes and syndromes of nadal dysgenesis, the method is widely ed, *e. g.* by Jacobs, Harnden, Court Brown, ldstein & Close (1960), Nowakowski, nz, Bergman & Reitalu (1960) and Hun-ford, Donnelly, Nowell & Beck (1960). In order to determine the sex chromatin this method, ordinary blood smears are ined with the usual May-Gruenwald-Giemsa stain and the granulocytes are then examined for the presence of so-called drumsticks, *i. e.* small round or oval shafted nuclear appendages. If six granulocytes with drumsticks are found, the sex chromatin is considered to be female and the counting can cease. In normal females and in patients with chromatin-positive Klinefelter's syndrome, six cells with drumsticks are usually found among 100—500 granulocytes examined.

If no drumsticks are found, the sex chromatin is considered male. Some workers, *e. g.* Kosenow & Scupin (1956) claim that one or two cells containing drumsticks can be found in normal males, whereas others, *e. g.* Wiedemann (1958) assert that even one drumstick strongly suggests that the sex chromatin is female. It is agreed that in cases in which no drumsticks are found, 500 granulocytes must be inspected to ensure the diagnosis of male sex chromatin. Some workers (Weidemann 1960, Maclean 1960) often count considerably more cells. However it is evident that in some patients a large number of cells have to be inspected in order to ensure a reliable result.

The number of granulocytes in an ordi-

nary blood smear varies a great deal. The white cell count and the incidence of different kinds of leukocytes is one variable. Another factor, which may be more important in dealing with healthy subjects, is the technique used in making the smears. Thick smears contain more cells than do thin ones. In the author's experience certain slides do not contain more than 200—400 granulocytes, whereas others may contain more than 1000. The leukocytes are usually spread uniformly over the whole slide, a higher concentration only being found along the edges of the film. However, these leukocytes along the edges are often damaged and cannot be used in the search for drumsticks.

Thus, the finding and inspection of a large number of granulocytes is a laborious task. As the cells have to be studied under a high-power oil-immersion lens, the search is slow and tiring for the eyes. The average time required for a reliable determination of the sex chromatin in normal males and in patients with a male chromatin pattern is about 15—60 minutes when ordinary blood smears are used. In routine work this time must be considered far too long. The use of a concentrated suspension of leukocytes for making smears considerably facilitates this work.

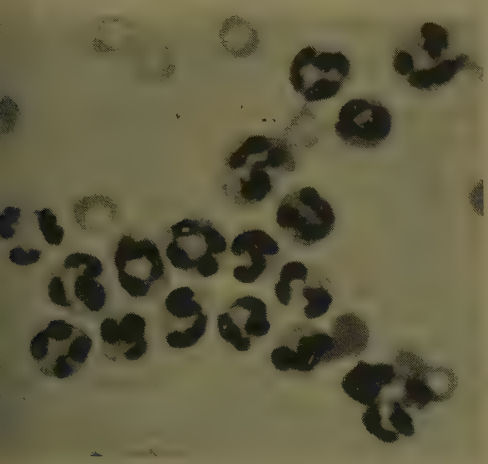
Several good methods for making concentrated leukocyte suspensions are in use (Klima 1949, Klima, Beyreder & Herzog 1956, Nordquist, Ryttinger & Ljunggren 1960, Spriggs & Alexander 1960). All these methods, which include either some hours' standing or the formation of a buffy coat seem to damage the fine structure of the granulocytes. As drumsticks in the polymorphonuclear cells are difficult to distinguish from other nuclear appendages even in good whole-blood smears, it is desirable to find a method that damages the cells as little as possible. The technique described in this paper has been developed to make concentrated leukocyte smear preparations to be used for determining the sex chromatin.

THE TECHNIQUE

Approximately 4 ml of venous blood is wit drawn into a centrifuge tube containing citra Immediately afterwards, 0.2 ml of Bacto-Phyt hemagglutinin (Difco Laboratories) is added a mixed in by shaking. The tube is placed in ic cold water and allowed to cool for 20 minutes. T tube is then centrifuged at about 500 r.p.m. for 2- minutes at + 4° C. After this, most of the r cells will have spun down. Above the red ce there is a layer of plasma containing the majori of the white cells originally present. The plasr layer is carefully aspirated off and poured into small centrifuge tube and centrifuged at 700 r.p. for 6 minutes at room temperature. This causes t white cells and the remaining erythrocytes sediment forming together a small reddish pel on the bottom of the tube. The supernatant liqu is again sucked off, leaving behind only one drop which the cells are resuspended by flicking the tu A drop of this thick suspension is then pipetted or a slide and an ordinary smear is made. The sme is dried and stained in the usual way by the Ma Gruenwald-Giemsa method.

COMMENTS

The amount of blood need not be consta Any amount exceeding 2 ml will do. F practical reasons, less than 2 ml is inco venient, as the amount of plasma is then le than 1 ml, and thus pipetting becomes mo tricky. The time required for the red cells sediment varies from one patient to anoth The tube should be inspected at least or after 2 minutes of centrifugation. If the ce trifugation is too prolonged, the white ce are lost in the mass of red cells or form buffy layer on the top of the red cells. Su granulocytes are not quite suitable for t detection of drumsticks. Pipetting should carried out carefully when the leukocy plasma suspension is being separated fr the red cell mass. If this is done too rapid many red cells will be drawn into the pipe and the proportion of red cells on the fi slide will be unnecessarily increased. T whole procedure takes about 70 minut Several specimens can, of course, be hand at one time. If the blood samples are left stand for a short time before being fr tionated, this will do no harm, but a



. 1. Leukocytes in a smear preparation from a centrated leukocyte suspension. May-Gruenwald-Giemsa stain. X 750.

riod of time over one hour will damage granulocytes.

Slides made by this technique contain usands of leukocytes. The cells are ually well and uniformly spread out, espe-lly if the smear is thin. When a high-wer oil-immersion lens is used, usually -20 leukocytes are seen in every field.) leukocytes can easily be inspected in -5 minutes. Figs. 1 and 2 show some ical views.

As far as the author is aware, no workers ling with sex chromatin have published nethod similar to the one described. Mac-n (1960) uses a method that gives about same result in the end, but with a differ-technique. The present method consider-y facilitates the search for drumsticks in nulocytes and the quality of the cells is od. The technique, including two stages of trifugation, is of course much more labo-us than an ordinary blood smear prepara-n. Samples cannot be sent long distances ause of the harmful effect of standing on cells. Therefore, the method is suitable y for laboratories in which numerous de-minations of sex chromatin are made.

SUMMARY

This paper deals with the determination of sex chromatin by the hematomorphological method. The advantages and disadvantages of using ordinary blood smear preparations are discussed.

The method described provides slides that contain a large number of leukocytes. The morphological quality of the cells is good. This greatly facilitates the search for and examination of polymorphonuclear leukocytes for the determination of drumsticks. It thus reduces the time required for a proper diagnosis of sex chromatin.

REFERENCES

Barr, M. L. & Bertram, E. G.: A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellites during accelerated nucleoprotein synthesis. Nature *163*, 676, 1949.

Davidson, W. M. & Smith, D. R.: A morphological sex difference in the polymorphonuclear neutrophil leukocytes. Brit. Med. J. *2*, 6, 1954.

Efrati, P. & Rosenszajn, L.: The morphology of buffy coat in normal human adults. Blood *16*, 1012, 1960.

Hungerford, D. A., Donnelly, A. J., Nowell, P. C. & Beck, S.: The chromosome constitution of a human phenotypic intersex. Am. J. Human Genet. *11*, 215, 1960.

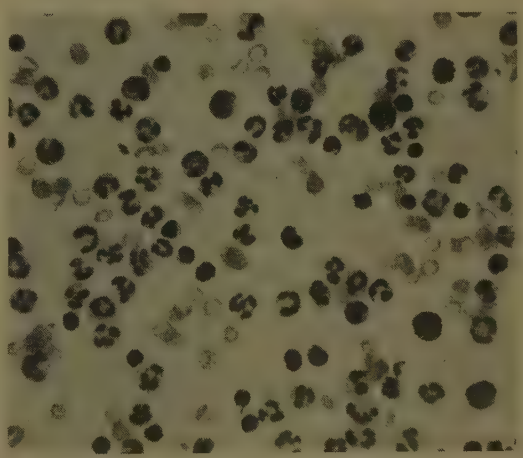


Fig. 2. Same method as in Fig. 1. X 300.

Jacobs, P. A., Harnden, D. G., CourtBrown, W. M., Goldstein, J., Close, H. G., MacGregor, T. N., Maclean, N. & Strong, J. A.: Abnormalities involving the X chromosome in women. Lancet *I*, 1213, 1960.

Klima, R., Zur Methodik der morphologischen Blutuntersuchung im Leukozytenkonzentrate. Wien. med. Wchnschr. *99*, 358, 1949.

— Beyreder, J. & Herzog, E.: Das Leukozytenkonzentrat als einfache und ergiebige Methode für die hämatologische Diagnostik. Wien. med. Wchnschr. *106*, 809, 1956.

Klinger, H. P. & Ludwig, K. S.: A universal stain for the sex chromatin body. Stain Techn. *32*, 235, 1957.

Kosenow, W. & Scupin, R.: Die Bestimmung des Geschlechtes mit Hilfe einer Kernanhangsformel der Leukozyten. Acta haemat. *15*, 349, 1956.

Maclean, N.: Personal communication, 1960.

Moore, K. L. & Barr, M. L.: Smears from the oral mucosa in the detection of chromosomal sex. Lancet *II*, 57, 1955.

Nordquist, P., Ryttinger, L. & Ljunggren, M.: simple method of preparing leukocyte susp sions. Scandinav. J. Clin. & Lab. Investigat *12*, 252, 1960.

Nowakowski, H., Lenz, W., Bergman, S. & R talu, J.: Chromosomenbefunde beim ech Klinefelter-Syndrom. Acta endocrinol. *34*, 4 1960.

Spriggs, A. I. & Alexander, R. F.: An albu gradient method for separating the differ white cells of blood, applied to the conc tration of circulating tumour cells. Nature *1* 863, 1960.

Wiedemann, H.-R.: The result of haematologi determination of the genetic sex in disturban of sexual development. Symposium on Nuck Sex. London, William Heinemann Medi Books Ltd. p. 102, 1958.

— Über die Zellkernmorphologische Geschlech diagnostik und ihre Bedeutung für die He kunde. Medizinische *33/34*, 1460, 1959.

— Personal communication, 1960.

ndinav. J. Clin. & Lab. Investigation 529, 13, 1961.

A SOLUTION FOR TESTING THE CLEANESS OF BLOOD PIPETTES

By J. LEHMANN

From the Central Laboratory for Clinical Chemistry, Sahlgren's Hospital, Gothenburg, Sweden.

(Received for publication January 19, 1961)

The validity of most analyses depends to reat extent on the accuracy of the volume asured of the sample to be analyzed. ring the last decade, micro- and ultra-cro analyses have gained ground in clinical mistry. Beside the calibration of the cropipettes and the pipetting technique d, the cleanness of the pipettes is a deter-ning factor for the accuracy. When using ter instead of saline as a rinsing solution er emptying a blood pipette, deposits of oteins and other biological material occur ularly on the inside of the pipette after ne use. These deposits are not visible to naked eye. The following solution will nediately color such deposits with a ght red color:

chsin (basic)	20 g
r. conc. (approx. 91 per cent v/v)	180 g (or c:a 220 ml)

The technique for controlling the clean-s of the pipettes with this solution is as ows: A rubber tube is attached to the ette and the pipette is filled with the solu-tion by suction. The solution is blown out and the pipette rinsed several times (5—10) with water. The pipette can also be attached to a water suction pump and a little of the solution drawn through the pipette. After half a minute the pipette is rinsed with water by dipping the tip of the pipette several times in water. Any color on the outside of the pipette is wiped of with cotton or filter paper. If the pipette is clean, no color will be seen on the inside, if not a red color will show the position of the deposits. After rinsing with a convenient rinsing solution the test should be repeated.

Experience has shown that even several washings with hot bichromate-sulphuric acid will not always clean up a dirty pipette. It should be mentioned that similar deposits will be found in glass electrodes rinsed with distilled water, and that the accuracy of the electrodes can be impaired by these deposits — often demonstrated by the difficulty in getting stable pH-values. The electrodes are best cleaned with a 0.1 per cent solution of pepsin in 0.01 n hydrochloric acid.

Letters to the Editors

Scandinav. J. Clin. & Lab. Investigation 530, 13, 1961.

SIMPLE SYNERESIS PROCEDURE FOR FIBRINOGEN ASSAY

The method for determination of plasma fibrinogen, described by Jacobsson (1955), gives reliable results. However, the syneresis procedure included in the method is a delicate and time-consuming operation. Here, a simple syneresis procedure is described.

The diluted plasma sample is clotted with thrombin in an ordinary test tube (internal diameter 13 mm). The final volume of the clotting mixture is 1.6 ml. After incubation for one hour at room temperature, the clot is carefully poured on a cloth and allowed to compact under its own weight. The cloth has been moistened with saline, and is placed on several layers of coarse filter paper. When the volume of the clot is reduced to about 1/2 of its original size, a glass slide (2.5 7.5 cm) is pressed firmly against it. A th fibrin film which adheres to the glass su face is formed, and the slide is then tran ferred to a beaker with saline. The clot readily loosened by moving the slide up a down in the saline, and the clot is thorough washed. Finally, the fibrin film is wound a small spatula, and excess fluid is remov by pressing it against a dry cloth. The cl is then dissolved and its protein conte determined as described by Jacobsson.

REFERENCES

Jacobsson, K.: Studies on fibrinogen. Scandin J. Clin. & Lab. Investigation 7, Suppl. 14, 19

The Institute for
Thrombosis Research,
Rikshospitalet, Oslo, Norway
March 2, 1961.

H. C. God

ndinav. J. Clin. & Lab. Investigation 531, 13, 1961.

PHOSPHOTUNGSTIC ACID AS PRECIPITATING AGENT IN THE DETERMINATION OF SPINAL FLUID PROTEIN BY THE LOWRY METHOD

In a previous communication (Svensmark 58) it was shown that a fixed correction ue for color-producing non-protein sub- nces (Daughaday, Lowry, Rosebrough Fields 1952) gives rise to considerable or in the determination of spinal fluid otein. The correction values varied from nple to sample, probably due to varying ntents of drugs, and it was recommended t the protein determination be corrected the color produced by a deproteinized quot of the sample. Daughaday *et al.* 952) used trichloracetic acid as precipita- g agent and in the modification reported eviously (Svensmark 1958) perchloric d was used. Both have the disadvantage t certain glycoproteins (mucoproteins) not precipitated resulting in too low pro- n values. A more complete precipitation s obtained with phosphotungstic acid:

Fifty μl of 9 per cent phosphotungstic acid 2 N HCl were added to 0.5 ml of the nal fluid sample in a centrifuge tube $\times$ 50 mm). After careful mixing the tube s allowed to stand for 1 hour before cen- fugation for 15 minutes at about 6,000 g. the supernatant, two samples of 0.2 ml re taken for analysis which was per- med as described previously.

The protein content of 51 spinal fluid nples was determined and corrected for color obtained from aliquots deprote- zed with perchloric acid and phospho- tungstic acid respectively. With perchloric acid the average spinal fluid protein was 0.33 $\pm$ 0.02 mg/ml (range: 0.06—1.03) and with phosphotungstic acid 0.38 $\pm$ 0.02 mg/ml (range: 0.10—1.10). The difference of 13 per cent of the total protein corresponds to the finding of 15 per cent perchloric acid soluble mucoprotein in spinal fluid (Bauer 1957).

The necessity of correcting the protein determination for color-producing non-protein substances was emphasized by the finding of Zondag & van Boetzelaer (1960) that several drugs (sulfonamide compounds, antibiotics and especially salicylic acids) produced large errors in the Lowry method when the extinction produced by the deproteinized sample was neglected.

REFERENCES

Bauer, H.: Zur Frage der Identität der Liquorproteine mit den Eiweisskörpern des Blutserums. Deutsche Ztschr. f. Nervenh. *176*, 126, 1957.

Daughaday, W. H., Lowry, O. H., Rosebrough, H. J. & Fields, W. S.: Determination of cerebrospinal fluid protein with the Folin phenol reagent. J. Lab. & Clin. Med. *39*, 663, 1952.

Svensmark, O.: Determination of protein in cerebrospinal fluid. A comment on the Lowry method. Scandinav. J. Clin. & Lab. Investigation *10*, 50, 1958.

Zondag, H. A. & van Boetzelaer, G. L.: Determination of protein in cerebrospinal fluid. Sources of error in the Lowry method. Clin. chim. Acta. *5*, 155, 1960.

e Institute of Neurophysiology
iversity of Copenhagen, Denmark
rch 24, 1961.

O. Svensmark.

Scandinav. J. Clin. & Lab. Investigation 532—533, 13, 1961.

THE RECOVERY OF THROMBIN FROM PLASMA

Sternberger and coworkers (1951, 1952, 1956) reported that small amounts of thrombin could be recovered from normal plasma. Larger amounts were recovered from the plasma of patients with postoperative complications and of rabbits with experimental thrombosis. They suggested that thrombin is continually formed by *in vivo* coagulation in normal individuals, and that the formation is increased in patients with thrombosis. Some of the thrombin is adsorbed to fibrin, and some is neutralized through the formation of an inactive complex with anti-thrombin. They apparently recovered the thrombin from this complex.

These findings, if confirmed, are extremely important. First, the presence of thrombin in normal plasma would indicate that a slow continuous coagulation takes place in the circulation of normal individuals. Still far from proved, such a hypothesis is frequently suggested, and several authors list Sternberger's findings as evidence (Copley 1954, Roos 1957, Lasch, Mechelke & Nusser 1957). Second, the presence of increased amounts of thrombin in conditions which predispose to thrombosis may suggest that thrombin plays an important role in the early phase of thrombus formation, and also that Sternberger's test might disclose candidates for thrombosis.

In reviewing the arguments in favor of continuous *in vivo* coagulation, we were forced to take a stand for, or against Sternberger's claims. We are not aware of any attempts to confirm the findings. Therefore, we repeated the key experiments.

Sternberger (1952) used the following technique. Citrated plasma is diluted with an equal of saline, and the pH is adjusted to 11.3 with sodium hydroxide, all at a temperature of 1° C. This supposedly dissociat the thrombin-antithrombin complex. Aft two minutes, an equal volume of 50 per ce ethanol is added to prevent reassociation the components, and the pH is returned 7.2 with hydrochloric acid. The resulti suspension of precipitated proteins, still 25 per cent ethanol, is tested for thromb activity on a substrate of oxalated plasma fibrinogen. Fibrinogen is unable to form ordinary clot at this concentration of etha (final concentration 12.5 per cent), but t appearance of granules was taken to indic clotting. The "clotting time" or the "gran appearance time" was therefore used measure the recovered thrombin.

Following this technique, we prepar suspensions from normal plasma which p duced a granular precipitate when mix with human plasma or bovine fibrinogen 18° C. However, the granulation did form in plasma at 37° C; in fact, the gr ulation which had formed at 18° C d appeared at 37° C. Therefore, the endpoi *i. e.* the "clotting time", could have be fibrinogen precipitated by ethanol in the t material, and not fibrin clotted by thromb To settle this problem, we centrifuged last mixture after the granulation had f med. The sediment dissolved rapidly in sal at 18° C, and clotted solidly after addition thrombin. The supernatant was nearly f of fibrinogen. We also found that a mixt of equal parts of 50 per cent ethanol citrated saline produced a granular p cipitate when added to the substrate, though it took somewhat longer to form was not as coarse.

The suspension prepared from plas contains fibrinogen which has been com tely precipitated by the ethanol. Fibrin c

clumps should nevertheless occur if the spension also contained free of recovered rombin. However, clots were not observed, fact which suggests that it may not contain rombin. As a further test, we added an ual volume of commercial bovine thrombin Topostasine, Hoffmann-La Roche, Switzer-nd) diluted in saline. This resulted in the rmation of coarse fibrin clumps which were ot soluble at 37° C and differed markedly om the fine granular precipitate described ove. With as little as 0.3 NIH units per l thrombin solution, this change, which we lieve is due to coagulation, occurred in 60 conds. Similar results were obtained with ovine thrombin in 25 per cent ethanol and ith human thrombin prepared by Stern-erger's method (1948). This observation onfirms that the suspension did not contain rombin.

Sternberger & Maltaner (1951) added rombin prepared by Sternberger's method 1948) to serum, waited for complete in-ctivation, and then recovered the thrombin uantitatively by the method describer. The rombin was measured by the "granule ppearance time". We have repeated this ex-eriment many times, and have always failed recover any thrombin which could pro-uce the coarse, irreversible fibrin clumps of rdinary thrombin.

From these observations we must reject ternberger's claim that normal plasma con-ins thrombin which can be recovered by is method. We believe that his assay is nspecific, and that granulation of the sub-rate is caused by ethanol precipitation of brinogen. It therefore does not measure rombin.

Reich & Sternberger (1956) found that ore thrombin could be recovered from the plasma of patients with postoperative complications than from normal individuals. Since we have failed to demonstrate any thrombin in normal plasma, we have not repeated this work. Their patients also had high concentrations of fibrinogen which may have influenced the assay. Thus, we found that granulation occurred more rapidly if we added "thrombin" recovered from plasma than that from the same patient's serum. We therefore believe that the assay is influenced by unspecific effects of slightly denatured proteins, especially fibrinogen.

Our experiments do not rule the possibility that plasma may contain thrombin, nor that continuous *in vivo* coagulation may be a normal phenomenon. However, if our observations and conclusions are correct, Sternberger's observations should no longer be used as evidence in favour of the occurrence of thrombin in normal plasma.

REFERENCES

Copley, A. L.: Thrombosis and thrombo-embolization in the blood capillaries. Proc. I int. Conf. Thrombosis and Embolism in Basel 1954. Benno Schwabe, Basel 1955, p. 452.

Lasch, H. G., Mechelke, K. & Nusser, E.: Über Änderungen der Gerinnungsfaktoren Prothrombin und VII bei hyper- und hypozirkulatorischen Kreislaufumstellungen der Katze. Deutsches Arch. f. klin. Med. *204,* 1, 1957.

Reich, T. & Sternberger, L. A.: Postoperative thrombin production Surg., Gynec. & Obst. *102,* 463, 1956.

Roos, J.: Blood coagulation as a continuous process. Thromb. Diath. haem. *1,* 471, 1957.

Sternberger, L. A.: Haemostasis with an easily prepared stable thrombin solution. J. Clin. Pathol. *1,* 229, 1948.

— Preliminary clinical evaluation of thrombin recovery test. J. Am. M. A. *150,* 1591, 1952.

— & Maltaner, F.: The recovery of preformed thrombin from circulating plasma. Science *114,* 414, 1951.

nstitute for Thrombosis Research,
ikshospitalet, Oslo, Norway

H. C. Godal,
R. Hasselback,
P. F. Hjort.

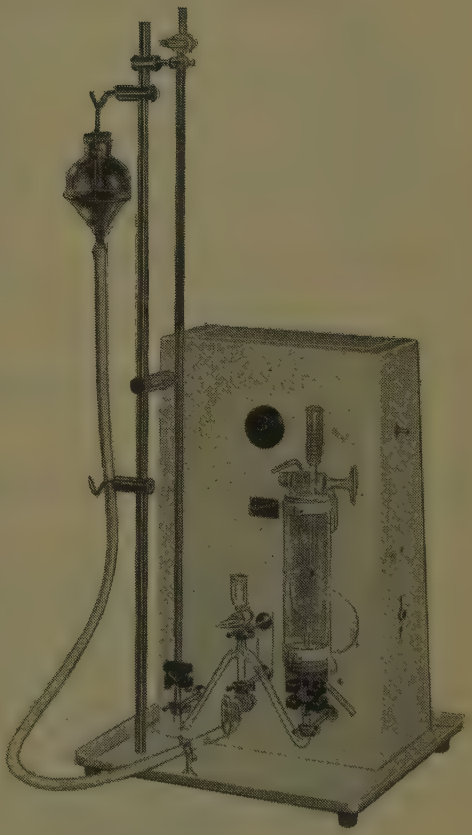
pril 25, 1961.

easier . . . faster . . . cleaner determinations

with our new magnetically agitated

VAN SLYKE APPARATUS

CO_2 in plasma and O_2 in whole blood can be extracted within the standard time of 3 minutes. Meanwhile the technician can load the reaction pipette funnel for the next determination. Magnetic agitation, minimising noise, vibration and risk of broken glass.



- ★ No shaking parts — no glass fatigue.
- ★ Easy quiet operation.
- ★ Trouble-free magnetic stirring.
- ★ Standard sized glassware — ready replacements.
- ★ Rapid dismantling for cleaning.
- ★ Large stopcocks accurately ground to retain vacuum — can be removed for greasing without dismantling the other glassware.
- ★ Large mercury tray — easy-clean surface.
- ★ Metal stand — rust-proofed and attractive.

Ask for details of this and the

APPAPORT APPARATUS

new micro method for blood gas analysis. Only 1 ml sample of plasma or serum required.

Gallenkamp **SUPPLY THE WORLD's LABORATORIES**

A. GALLENKAMP & CO. LTD., SUN STREET, LONDON, E. C. 2 ENGLAND

APPOINTED AGENT FOR NORWAY

NERLIENS KEMISK-TEKNISKE AKTIESELSKAP

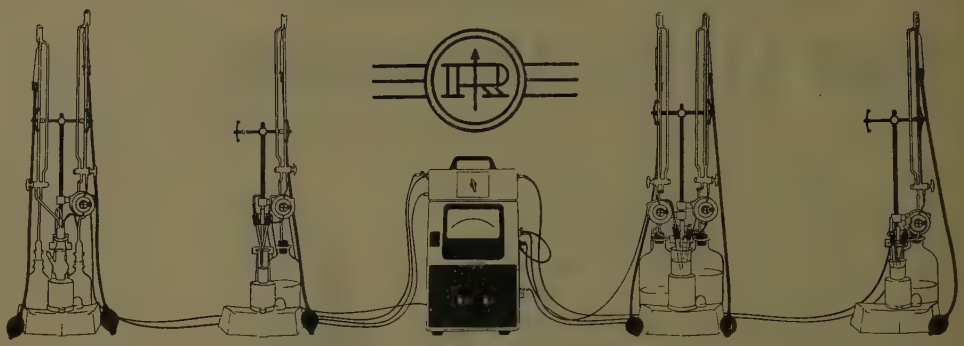
Tollbodgt. 32, Oslo

APPOINTED AGENT FOR SWEDEN

SIGURD HOLM AB

Olshammarsgt. 89, Bandhagen. Tel: 86 82 32, 86 82 42, 86 82 80

RADIOMETER AUTOMATIC TITRATOR, type TTT 1



TTA1/KF	TTA1	TTT1/ELS12	TTA1	TTA1
KARL FISCHER TITRATIONS	BLOOD SUGAR TITRATIONS		ACID BASE TITRATIONS	CHLORIDE TITRATIONS

— Ideal also for all other potentiometric titrations inclusive of titrations at constant pH, e.g. determinations of choline esteraze

RADIOMETER

72, EMDRUPVEJ, COPENHAGEN, DENMARK

D.L. büretten

Dansk Laboratorieudstyr A/S

3, Ryesgade *Luna 2600 København N

BB KONSTRIKTIONSPIPETTER..

Hver pipette er omhyggeligt udmålt og indstiller sig automatisk på sit rumfang. Pipetterne leveres emballeret i klare plasticrør, der yder god beskyttelse under opbevaringen.

BB konstriktionspipetter leveres i størrelserne 1—3000 cmm.

BIE & BERNTSEN

PILESTRÆDE 35, KØBENHAVN K

Mettler

Analysevekter og presisjonsvekter

LEYBOLD

Vakumpumper og vakumutstyr

Radiometer elektrokjemiske måleapparater

REICHERT WIEN

Mikroskop og optisk utstyr

Representant for Norge

NERLIENS KEMISK-TEKNISKE AKTIESELSKAP

Vˇ. Torgv. 5, Bergen	Tollbodgt. 32, Oslo	Kjøbmannsgt. 34, Trondheim
tlf. 15 981	Sentralbord 41 73 43	tlf. 20 469

Stavolt

Elektronisk spændingsstabilisator til papir-elektroforese. Tilsluttes 220 volt vekselstrø 50—60 perioder, og giver en vel reguleret stabiliseret spænding og/eller strøm og derv yderst reproducérbare arbejdsbetingelser.

- 4 indstillingsmuligheder: 0—250 volt, 250 500 volt, 2—30 milliampère og 30—50 mil ampère.
- Maximalvariation ± 1 % ved netspændin variationer ± 10 %.
- Udvendige mål: 260 × 200 × 215 m/m.

STRUERS

H. Struers chemiske laboratorium

KØBENHAVN C. 1402 · AARHUS 3 1611 · ODENSE 12360

PHEROGRAF

ORIGINAL FRANKFURT

Högspänningselektrofores för analytisk- och mikropreparativ separering.

Två separationskammare 350 × 400 mm med separat högspänningsaggregat 0—3000 V, 0—150 mA. Inbyggt kylaggregat 450 kcal/h med automatisk kylreglering och kylslingor i varje kammare.

Elektrofores kan utföras vid alla pH-värden på filtrerpapper, filtrerkartong, stärkelse och cellulosapulver.

främst för laboratoriet —

Rudolph Grave AB

ETABLERAD 1874

STOCKHOLM	GÖTEBORG	MALMÖ
TEL 23 37 80	TEL 27 51 35	TEL 38125

NOTICE TO AUTHORS

Manuscripts should be typewritten in English, double spaced throughout.

Tables, diagrams and other inserts should be sent on separate sheets, bearing the author's name. The legends for the figures should be typed separately, and the figures should be comprehensible without reference to the text.

All figures and illustrations are to be submitted in such a form as to permit photographic reproduction without retouching or re-drawing. They should be trimmed to the smallest area and be approximately twice the size of the finished block. Diagrams should be in Indian ink and should be drawn on plain white paper, tracing paper, Bristol board or faintly blue-lined paper. For photographs glossy prints, unmounted and not retouched, are required. Lines in line drawing, letters, numbers etc. should be of dimensions to stand reduction, usually to one-half or one-third. Unsuitable figures will be re-drawn by the press. All expenses of re-drawing, will be charged to the author.

The introduction should contain a clear statement of the purpose of the investigation and its relation to other works in the same field, but extensive reviews must be avoided.

The experimental procedure must be clearly described. However, methods should be given in extenso only if they represent a new approach.

Every paper should end with a brief summary. References to the literature should be listed alphabetically at the end of the paper, and the names of the authors should be mentioned in the text with the year of publication in brackets. The bibliography should conform to the style used in this journal: The author's name, initials, title of paper, abbreviation of journal, volume, page and year; in the case of books, the title in full, with the place and year of publication, and page.

No paper should exceed 12 printed pages. — Short reports of important findings, occupying not more than 2 printed pages, will be accepted for prompt publication in the form of *Letters to the Editors*.

CONTENTS

S. JARNUM and N. A. LASSEN: Albumin and transferrin metabolism in infectious and toxic diseases 357
E. GJONE: Inhibitory action of serum lecithin on the hemolytic activity of serum lysolecithin *in vitro* 369
L. KRAMER JAKOBSEN: Quantitative determination of urinary glucose using glucose oxidase and peroxidase 374
T. ASTRUP and P. OLLENDORFF: Estimation of thromboplastin activation in plasma 377
L.-G. ALLGRÉN, K. E. FUNKE and B. NAUCKHOFF: Fluorometric determination of tryptamine in urine with the Zeiss ultraviolet spectrophotometer-fluorometer 390
K. EKBOM and B. WETTERDAL: Paper-electrophoretic studies on human semen with special interest in the distribution of administered Zn^{65} 396
K. KRISTOFFERSEN: An improved method for the estimation of small quantities of alkali-resistant hemoglobin in blood 402
E. S. OLESEN: A fibrinolytic system in human plasma activated by peptone or acid polysaccharides 410
Å. HOLMGÅRD: On the determination of carbonic anhydrase in biological material 416
A. CARLSTEN, B. HALLGREN, R. JAGENBURG, A. SVANBORG and L. WERKÖ: Myocardial metabolism of glucose, lactic acid, amino acids and fatty acids in healthy human individuals at rest and at different work loads 418
I. FALBE-HANSEN: Spectrophotometric studies on denatured globin hemichrome 429
P. BJÖRNTORP and K. CRAMÉR: Influence of cholesteryl esters from human serum on serum cholesterol determination with the modified Theorell method 434
S. BEVEGÅRD, B. JONSSON and I. KARLÖF: Percutaneous technique for transseptal left heart catheterization *via* the right femoral vein 439
AA. DRIVSHOLM and S. MADSEN: The influence of treatment with sodium salisylate on the serum glutamic oxalacetic transaminase activity 442
S. JARNUM: The ^{131}I-polyvinylpyrrolidone (^{131}I-PVP) test in gastrointestinal protein loss 447
S. JARNUM: Fecal ^{131}I-output after intravenous injection of ^{131}I-labelled human serum albumin in normo- and hypoproteinemic subjects 462
E. BERNE, L. HALLBERG and S. E. LINDELL: A method for recording rapid changes in radiation intensity applied to cardiovascular studies 476
H. E. REFSUM: Hypokalemic alkalosis with paradoxical aciduria during artificial ventilation of patients with pulmonary insufficiency and high plasma bicarbonate concentration 481
H. CARSTENFORS, H. ELIASCH and E. HULTMAN: Estimation of the splanchnic blood flow in man by the bromsulphalein (BSP) method; the effect of continuous variation of the peripheral plasma concentration of BSP 489
E. HULTMAN and H. CARSTENFORS: Effect of injection of hypertonic glucose on splanchnic blood flow and oxygen consumption 503
H. CARSTENFORS, H. ELIASCH and E. HULTMAN: Effects of ingestion of hyperosmotic glucose solution on the splanchnic circulation in normal subjects and in partially gastrectomized patients reacting with circulatory collapse 512

Technique and practical problems:

A. DE LA CHAPELLE: A simple technique for concentrating granulocytes for the determination of chromatin sex 525
J. LEHMANN: A solution for testing the cleaness of blood pipettes 529

Letters to the editors:

H. C. GODAL: Siimple syneresis procedure for fibrinogen assay 530
O. SVENSMARK: Phosphotungstic acid as precipitating agent in the determination of spinal fluid protein by the Lowry method 531
H. GODAL, R. HASSELBACK and P. F. HJORT: The recovery of thrombin from plasma 532

DISTRIBUTED BY EJNAR MUNKSGAARD, COPENHAGEN, DENMARK

PRINTING ARRANGEMENTS BY OSLO UNIVERSITY PRESS, OSLO, NORWAY

PRINTED BY A. W. BRØGGERS BOKTRYKKERI A/S, OSLO